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(54) Title: REDIRECTION OF CELLULAR IMMUNITY BY RECEPTOR CHIMERAS

(57) Abstract

Disclosed is a method of directing a cellular response in a mammal by expressing in a cell of the mammal a chimeric re-ceptor which causes the cells to specifically recognize and destroy an infective agent, a cell infected with an infective agent, a tu-mor or cancerous cell, or an autoimmune-generated cell. Also disclosed are cells which express the chimeric receptors and DNA encoding the chimeric receptors.

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REDIRECTION OF CELLULAR IMMUNITY BY RECEPTOR CHIMERAS
Field of the Invention

The invention concerns functional T cell receptor,
5 Fc receptor, or B cell receptor chimeras which are
capable of redirecting immune system function. More
particularly, it concerns the regulation of lymphocytes,
macrophages, natural killer cells or granulocytes by the
expression in said cells of chimeras which cause the
10 cells to respond to targets recognized by the chimeras.
The invention also concerns functional T cell receptor,
Fc receptor, or B cell receptor chimeras which are
capable of directing therapeutic cells to specifically
recognize and destroy either cells infected with a
15 specific infective agent, the infective agent itself, a
tumor cell, or an autoimmune-generated cell. More
particularly, the invention relates to the production of
T cell receptor or Fc receptor chimeras capable of
directing cytotoxic T lymphocytes to specifically
20 recognize and lyse cells expressing HIV envelope
proteins. The invention therefore provides a therapy for
diseases such as AIDS (Acquired Immunodeficiency
Syndrome) which are caused by the HIV virus.

Background of the Invention

25   T cell recognition of antigen through the T cell
receptor is the basis of a range of immunological
phenomena. The T cells direct what is called cell-
mediated immunity. This involves the destruction by
cells of the immune system of foreign tissues or infected
30 cells. A variety of T cells exist, including "helper"
and "suppressor" cells, which modulate the immune
response, and cytotoxic (or "killer") cells, which can
kill abnormal cells directly.

A T cell that recognizes and binds a unique
35 antigen displayed on the surface of another cell becomes

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activated; it can then multiply, and if it is a cytotoxic
cell, it can kill the bound cell.

Autoimmune disease is characterized by production
of either antibodies that react with host tissue or
5 immune effector T cells that are autoreactive. In some
instances, autoantibodies may arise by a normal T- and B-
cell response activated by foreign substances or
organisms that contain antigens that cross react with
similar compounds in body tissues. Examples of
10 clinically relevant autoantibodies are antibodies against
acetylcholine receptors in myasthenia gravis; and anti-
DNA, anti-erythrocyte, and anti-platelet antibodies in
systemic lupus erythematosus.

HIV and Immunopathogenesis
15   In 1984 HIV was shown to be the etiologic agent of
AIDS. Since that time the definition of AIDS has been
revised a number of times with regard to what criteria
should be included in the diagnosis. However, despite
the fluctuation in diagnostic parameters, the simple
20 common denominator of AIDS is the infection with HIV and
subsequent development of persistent constitutional
symptoms and AIDS defining diseases such as a secondary
infections, neoplasms, and neurologic disease.
Harrison's Principles of Internal Medicine, 12th ed.,
25 McGraw Hill (1991).

HIV is a human retrovirus of the lentivirus group.
The four recognized human retroviruses belong to two
distinct groups: the human T lymphotropic (or leukemia)
retroviruses, HTLV-1 and HTLV-2, and the human
30 immunodeficiency viruses, HIV-1 and HIV-2. The former
are transforming viruses whereas the latter are
cytopathic viruses.

HIV-1 has been identified as the most common cause
of AIDS throughout the world. Sequence homology between

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HIV-2 and HIV-1 is about 40% with HIV-2 being mor
closely related to some members of a group of simian
immunodeficiency viruses (SIV). See Curran, J. et al.,
Science, _329_:1357-1359 (1985); Weiss, R. et al., _Nature_,
5 _324_:572-575 (1986).

HIV has the usual retroviral genes (_env_, _gag_, and
pol) as well as six extra genes involved in the
replication and other biologic activities of the virus.
As stated previously, the common denominator of AIDS is a
10 profound immunosuppression, predominantly of cell-
mediated immunity. This immune suppression leads to a
variety of opportunistic diseases, particularly certain
infections and neoplasms.

The main cause of the immune defect in AIDS, has
15 been identified as a quantitative and qualitative
deficiency in the subset of thymus-derived (T)
lymphocytes, the T4 population. This subset of cells is
defined phenotypically by the presence of the CD4 surface
molecule, which has been demonstrated to be the cellular
20 receptor for HIV. Dalgleish et al., _Nature_, _312_:763
(1984). Although the T4 cell is the major cell type
infected with HIV, essentially any human cell that
expresses the CD4 molecule on its surface is capable of
binding to and being infected with HIV.

25 Traditionally, CD4$^+$ T cells have been assigned the
role of helper/inducer, indicating their function in
providing an activating signal to B cells, or inducing T
lymphocytes bearing the reciprocal CD8 marker to become
cytotoxic/suppressor cells. Reinherz and Schlossman,
30 _Cell_, _19_:821-827 (1980); Goldstein et al., _Immunol. Rev._,
_68_:5-42, (1982).

HIV binds specifically and with high affinity, via
a stretch of amino acids in the viral envelope (gp120),
to a portion of the V1 region of the CD4 molecule located
35 near its N-terminus. Following binding, the virus fuses

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with the target cell membrane and is int rnalized. Once
internalized it uses the enzym reverse transcriptase to
transcribe its genomic RNA to DNA, which is integrated
into the cellular DNA where it exists for the life or the
5 cell as a "provirus."

The provirus may remain latent or be activated to
transcribe mRNA and genomic RNA, leading to protein
synthesis, assembly, new virion formation, and budding of
virus from the cell surface. Although the precise
10 mechanism by which the virus induces cell death has not
been established, it is felt that the major mechanism is
massive viral budding from the cell surface, leading to
disruption of the plasma membrane and resulting osmotic
disequilibrium.

15   During the course of the infection, the host
organism develops antibodies against viral proteins,
including the major envelope glycoproteins gp120 and
gp41. Despite this humoral immunity, the disease
progresses, resulting in a lethal immunosuppression
20 characterized by multiple opportunistic infections,
parasitemia, dementia and death. The failure of the host
anti-viral antibodies to arrest the progression of the
disease represents one of the most vexing and alarming
aspects of the infection, and augurs poorly for
25 vaccination efforts based upon conventional approaches.

Two factors may play a role in the efficacy of the
humoral response to immunodeficiency viruses. First,
like other RNA viruses (and like retroviruses in
particular), the immunodeficiency viruses show a high
30 mutation rate in response to host immune surveillance.
Second, the envelope glycoproteins themselves are heavily
glycosylated molecules presenting few epitopes suitable
for high affinity antibody binding. The poorly antigenic
target which the viral envelope presents, allows the host

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little opportunity for r stricting viral infection by
specific antibody production.

Cells infected by the HIV virus express the gp120
glycoprotein on their surface. Gp120 mediates fusion
5   events among CD4$^+$ cells via a reaction similar to that by
which the virus enters the uninfected cells, leading to
the formation of short-lived multinucleated giant cells.
Syncytium formation is dependent on a direct interaction
of the gp120 envelope glycoprotein with the CD4 protein.
10 Dalgleish et al., supra; Klatzman, D. et al., Nature,
312:763 (1984); McDougal, J.S. et al., Science, 231:382
(1986); Sodroski, J. et al., Nature, 322:470 (1986);
Lifson, J.D. et al., Nature, 323:725 (1986); Sodroski, J.
et al., Nature, 321:412 (1986).

15   Evidence that the CD4-gp120 binding is responsible
for viral infection of cells bearing the CD4 antigen
includes the finding that a specific complex is formed
between gp120 and CD4. McDougal et al., supra. Other
investigators have shown that the cell lines, which were
20 noninfective for HIV, were converted to infectable cell
lines following transfection and expression of the human
CD4 cDNA gene. Maddon et al., Cell, 46:333-348 (1986).

Therapeutic programs based on soluble CD4 as a
passive agent to interfere with viral adsorption and
25 syncytium-mediated cellular transmission have been
proposed and successfully demonstrated in vitro by a
number of groups (Deen et al., Nature, 3321:82-84 (1988);
Fisher et al., Nature, 331:76-78 (1988); Hussey et al.,
Nature 331:78-81 (1988); Smith et al., Science, 238:1704-
30 1707 (1987); Traunecker et al., Nature, 331:84-86
(1988)); and CD4 immunoglobulin fusion proteins with
extended halflives and modest biological activity have
subsequently been developed (Capon et al., Nature,
337:525-531 (1989); Traunecker et al. Nature, 339, 68-70
35 (1989); Byrn et al., Nature, 344:667-670 (1990);

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Zettlm issl et al., <u>DNA Cell Biol.</u> 9:347-353 (1990)).
Although CD4 immunotoxin conjugates or fusion proteins
show potent cytotoxicity for infected cells <u>in vitro</u>
(Chaudhary et al., <u>Nature</u>, 335:369-372 (1988); Till et

5   al., <u>Science</u>, 242:1166-1168 (1988)), the latency of the
immunodeficiency syndrome makes it unlikely that any
single-treatment therapy will be effective in eliminating
viral burden, and the antigenicity of foreign fusion
proteins is likely to limit their acceptability in

10   treatments requiring repetitive dosing. Trials with
monkeys affected with SIV have shown that soluble CD4, if
administered to animals without marked CD4 cytopenia, can
reduce SIV titer and improve <u>in vitro</u> measures of myeloid
potential (Watanabe et al., <u>Nature</u>, 337:267-270 (1989)).

15   However a prompt viral reemergence was observed after
treatment was discontinued, suggesting that lifelong
administration might be necessary to prevent progressive
immune system debilitation.

T Cell and Fc Receptors

20   Cell surface expression of the most abundant form
of the T cell antigen receptor (TCR) requires the
coexpression of at least 6 distinct polypeptide chains
(Weiss et al., <u>J. Exp. Med.</u>, 160:1284-1299 (1984);
Orloffhashi et al., <u>Nature</u>, 316:606-609 (1985); Berkhout

25   et al., <u>J. Biol. Chem.</u>, <u>J. Biol. Chem.</u>, 263:8528-8536
(1988); Sussman et al., <u>Cell</u>, 52:85-95 (1988)), the α/β
antigen binding chains, the three polypeptides of the CD3
complex, and ζ. If any of the chains are absent, stable
expression of the remaining members of the complex does

30   not ensue. ζ is the limiting polypeptide for surface
expression of the complete complex (Sussman et al., <u>Cell</u>,
52:85-95 (1988)) and is thought to mediate at least a
fraction of the cellular activation programs triggered by
receptor recognition of ligand (Weissman et al., <u>EMBO J.</u>,

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8:3651-3656 (1989); Frank et al., <u>Science</u>, <u>249</u>:174-177
(1990)). A 32kDa type I integral membrane homodimer, ʕ
(zeta) has a 9 residue extracellular domain with no sites
for N-linked glycan addition, and a 112 residue (mouse)
5 or 113 residue (human) intracellular domain (Weissman et
al., <u>Science</u>, 238:1018-1020 (1988a); Weissman et al.,
<u>Proc. Natl. Acad. Sci. USA</u>, <u>85</u>:9709-9713 (1988b)). An
isoform of ʕ called η (eta) (Baniyash et al., <u>J. Biol.
Chem.</u>, <u>263</u>:9874-9878 (1988); Orloff et al., <u>J. Biol.
10 Chem.</u>, <u>264</u>:14812-14817 (1989)), which arises from an
alternate mRNA splicing pathway (Jin et al., <u>Proc. Natl.
Acad. Sci. USA</u>, <u>87</u>:3319-3233 (1990)), is present in
reduced amounts in cells expressing the antigen receptor.
ʕ-η heterodimers are thought to mediate the formation of
15 inositol phosphates, as well as the receptor-initiated
programmed cell death called apoptosis (Merćep et al.,
<u>Science</u>, <u>242</u>:571-574 (1988); Merćep et al., <u>Science</u>,
<u>246</u>:1162-1165 (1989)).

        Like ʕ and η, the Fc receptor-associated γ chain
20 is expressed in cell surface complexes with additional
polypeptides, some of which mediate ligand recognition,
and others of which have undefined function. γ (gamma)
bears a homodimeric structure and overall organization
very similar to that of ʕ, and is a component of both the
25 mast cell/basophil high affinity IgE receptor, FcεRI,
which consists of at least three distinct polypeptide
chains (Blank et al., <u>Nature</u>, <u>337</u>:187-189 (1989); Ra et
al., <u>Nature</u>, <u>241</u>:752-754 (1989)), and one of the low
affinity receptors for IgG, represented in mice by
30 FcγRIIα (RA et al., <u>J. Biol. Chem. J. Biol. Chem.</u>,
<u>264</u>:15323-15327 (1989)), and in humans by the CD16
subtype expression by macrophages and natural killer
cells, $CD16_{TM}$ (CD16 transmembrane) (Lanier et al., <u>Nature</u>,
<u>342</u>:803-805 (1989); Anderson et al., <u>Proc. Natl. Acad.
35 Sci. USA</u>, <u>87</u>:2274-2278 (1990)) and with a polypeptide of

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unidentified function (Anderson et al., Proc. Natl. Acad. Sci. USA, 87:2274-2278 (1990)). Recently it has be n reported that γ is expressed by a mouse T cell line, CTL, in which it forms homodimers as well as γ-ζ and γ-η
5 heterodimers (Orloff et al., Nature, 347:189-191 (1990)).

The Fc receptors mediate phagocytosis of immune complexes, transcytosis, and antibody dependent cellular cytotoxicity (ADCC) (Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991); Unkeless et al., Annu. Rev.
10 Immunol 6:251-281 (1988); and Mellman, Curr. Opin. Immunol. 1:16-25 (1988)). Recently it has been shown that one of the murine low affinity Fc receptor isoforms (FcRγIIIB1) mediates internalization of Ig-coated targets into clathrin coated pits, and that another low affinity
15 receptor (FcrγIIIA) mediates ADCC through its association with one or more members of a small family of 'trigger molecules' (Miettinen et al., Cell 58:317-327 (1989); and Hunziker and Mellman, J. Cell Biol. 109:3291-3302 (1989)). These trigger molecules, T cell receptor (TCR)
20 ζ chain, TCR η chain, and Fc receptor γ chain, interact with ligand recognition domains of different immune system receptors and can autonomously initiate cellular effector programs, including cytolysis, following aggregation (Samelson et al., Cell 43:223-231 (1985);
25 Weissman et al., Science 239:1018-1020 (1988); Jin et al., Proc. Natl. Acad. Sci. USA 87:3319-3323 (1990); Blank et al., Nature 337:187-189 (1989); Lanier et al., Nature 342:803-805 (1989); Kurosaki and Ravetch, Nature 342:805-807 (1989); Hibbs et al., Science 246:1608-1611
30 (1989); Anderson et al., Proc. Natl. Acad. Sci USA 87:2274-2278 (1990); and Irving and Weiss, Cell 64: 891-901 (1991)).

In drawing parallels between the murine and human low affinity Fc receptor families, however, it has become
35 clear that the human FcRγIIA and C isoforms have no

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murine count rpart. In part because of this, their
function has yet to be defined.

Because humoral agents based on CD4 may have
limited utility _in vivo_, the inventors began to explore
5   the possibility of augmenting cellular immunity to HIV.
As a result they report the preparation of protein
chimeras in which the extracellular domain of CD4 is
fused to the transmembrane and/or intracellular domains
of T cell receptor, IgG Fc receptor, or B cell receptor
10 signal transducing elements. Cytolytic T cells
expressing chimeras which include an extracellular CD4
domain show potent MHC-independent destruction of
cellular targets expressing HIV envelope proteins. An
extremely important and novel component of this approach
15 has been the identification of single T cell receptor, or
Fc receptor, and B cell receptor chains whose aggregation
suffices to initiate the cellular response.

One particularly useful application of this
approach has been the invention of chimeras between CD4
20 and ζ, η, or γ that direct cytolytic T lymphocytes to
recognize and kill cells expressing HIV gp120.

Summary of the Invention

Although native T cell, B cell, and Fc receptors
are or can be highly complicated multimeric structures
25 not lending themselves to convenient manipulation, the
present invention demonstrates the feasibility of
creating chimeras between the intracellular domain of any
of a variety of molecules which are capable of fulfilling
the task of target recognition. In particular, the
30 formation of chimeras consisting of the intracellular
portion of T cell/Fc receptor zeta, eta, or gamma chains
joined to the extracellular portion of a suitably
engineered antibody molecule allows the target
recognition-potential of an immune system cell to be

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specifically redirected to the antigen recognized by the
extracellular antibody portion. Thus with an antibody
portion capable of recognizing some determinant on the
surface of a pathogen, immune system cells armed with the
5 chimera would respond to the presence of the pathogen
with the effector program appropriate to their lineage,
e.g., helper T lymphocytes would respond by cytotoxic
activity against the target, and B lymphocytes would be
activated to synthesize antibody. Macrophages and
10 granulocytes would carry out their effector programs,
including cytokine release, phagocytosis, and reactive
oxygen generation. Similarly, with an antibody portion
capable of recognizing tumor cells, the immune system
response to the tumor would be beneficially elevated.
15 With an antibody capable of recognizing immune cells
having an inappropriate reactivity with self
determinants, the autoreactive cells could be selectively
targeted for destruction. Although these examples draw
on the use of antibody chimeras as a convenient
20 expository tool, the invention is not limited in scope to
antibody chimeras, and indeed, the use of specific
nonantibody extracellular domains may have important
advantages. For example with an extracellular portion
that is the receptor for a virus, bacterium, or parasite,
25 cells armed with the chimeras would specifically target
cells expressing the viral, bacterial or parasitic
determinants. The advantage of this approach over the
use of antibodies is that the native receptor for
pathogen may have uniquely high selectivity or affinity
30 for the pathogen, allowing a greater degree of precision
in the resulting immune response. Similarly, to delete
immune system cells which inappropriately react with a
self antigen, it may suffice to join the antigen (either
as an intact protein, in the case of B cell depletion
35 therapies, or as MHC complex, in the case of T cell

depletion therapies) to intracellular zeta, eta or gamma
chains, and thereby affect the sp cific targ ting of the
cells inappropriately responding to self determinants.

        Another use of the chimeras is the control of cell
5 populations in vivo subsequent to other forms of genetic
engineering. For example, the use of tumor infiltrating
lymphocytes or natural killer cells to carry cytotoxic
principles to the site of tumors has been proposed. The
present invention provides a convenient means to regulate
10 the numbers and activity of such lymphocytes and cells
without removing them from the body of the patient for
amplification in vitro. Thus, because the intracellular
domains of the chimeric receptors mediate the
proliferative responses of the cells, the coordination of
15 the extracellular domains by a variety of aggregating
stimuli specific for the extracellular domains (e.g., an
antibody specific for the extracellular domain) will
result in proliferation of the cells bearing the
chimeras.
20   Although the specific embodiments of the present
invention comprise chimeras between zeta, eta or gamma
chains, or active fragments thereof (e.g., those
discussed below), any receptor chain having a similar
function to these molecules, e.g., in granulocytes or B
25 lymphocytes, could be used for the purposes disclosed
here. The distinguishing features of desirable immune
cell trigger molecules comprise the ability to be
expressed autonomously (i.e., as a single chain), the
ability to be fused to an extracellular domain such that
30 the resultant chimera is present on the surface of a
therapeutic cell, and the ability to initiate cellular
effector programs upon aggregation secondary to encounter
with a target ligand.

        At present the most convenient method for delivery
35 of the chimeras to immune system cells is through some

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form of genetic therapy. However r constituting immun
system c lls with chimeric receptors by mixture of the
cells with suitably solubilized purified chimeric protein
would also result in the formation of an engineered cell
5 population capable of responding to the targets
recognized by the extracellular domain of the chimeras.
Similar approaches have been used, for example, to
introduce the intact HIV receptor, CD4, into erythrocytes
for therapeutic purposes. In this case the engineered
10 cell population would not be capable of self renewal.

The present invention relates to functional
simplified T cell receptor, B cell receptor, and Fc
receptor chimeras which are capable of redirecting immune
system function. More particularly, it relates to the
15 regulation of lymphocytes, macrophages, natural killer
cells or granulocytes by the expression in said cells of
chimeras which cause the cells to respond to targets
recognized by the chimeras. The invention also relates
to a method of directing cellular response to an
20 infective agent, a tumor or cancerous cell, or an
autoimmune generated cell. The method for directing the
cellular response in a mammal comprises administering an
effective amount of therapeutic cells to said mammal,
said cells being capable of recognizing and destroying
25 said infective agent, tumor, cancer cell or autoimmune
generated cell.

In another embodiment, the method of directing
cellular response to an infective agent comprises
administering therapeutic cells capable of recognizing
30 and destroying said agent, wherein the agent is a
specific virus, bacteria, protozoa, or fungi. Even more
specifically, the method is directed against agents such
as HIV and Pneumocystis carinii.

Specifically the invention provides for a method
35 of directing cellular response to an HIV infected cell.

The method comprises administering to a patient an
effectiv amount of cytotoxic T lymphocytes, said
lymphocytes being capable of specifically recognizing and
lysing cells infected with HIV.

5   Thus, in one embodiment, there is provided
according to the invention a method for directing
cellular response to HIV infected cells, comprising
administering to a patient an effective amount of
cytotoxic T lymphocytes which are capable of specifically
10 recognizing and lysing cells infected with HIV.

In yet another embodiment is provided the chimeric
receptor proteins which direct the cytotoxic T
lymphocytes to recognize and lyse the HIV infected cell.
Yet another embodiment of the invention comprises host
15 cells transformed with a vector comprising the chimeric
receptors.

In yet another embodiment, the present invention
provides for an antibody against the chimeric receptors
of the invention.

20   In order to obtain cytotoxic T lymphocytes which
specifically bind and lyse cells infected with HIV, the
present inventors therefore attempted, and herein
receptor chimeras. These chimeric receptors are
functionally active and possess the extraordinary ability
25 of being able to specifically bind and lyse cells
expressing gp120.

It is an object of the present invention, then, to
provide for a method of treatment for individuals
infected with HIV. The present invention thus provides a
30 number of important advances in the therapy of AIDS.

These and other non-limiting embodiments of the
present invention will be apparent to those of skill from
the following detailed description of the invention.

In the following detailed description, reference
35 will be made to various methodologies known to those of

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skill in the art of molecular biology and immunology.
Publications and other materials setting forth such known
methodologies to which reference is made are incorporated
herein by reference in their entireties as though set
5 forth in full.

     Standard reference works setting forth the general
principles of recombinant DNA technology include Watson,
J.D. et al., Molecular Biology of the Gene, Volumes I and
II, the Benjamin/Cummings Publishing Company, Inc.,
10 publisher, Menlo Park, CA (1987); Darnell, J.E. et al.,
Molecular Cell Biology, Scientific American Books, Inc.,
Publisher, New York, N.Y. (1986); Lewin, B.M., Genes II,
John Wiley & Sons, publishers, New York, N.Y. (1985);
Old, R.W., et al., Principles of Gene Manipulation: An
15 Introduction to Genetic Engineering, 2d edition,
University of California Press, publisher, Berkeley, CA
(1981); Maniatis, T., et al., Molecular Cloning: A
Laboratory Manual, 2nd Ed. Cold Spring Harbor Laboratory,
publisher, Cold Spring Harbor, NY (1989); and Current
20 Protocols in Molecular Biology, Ausubel et al., Wiley
Press, New York, NY (1989).

DEFINITIONS

     By "cloning" is meant the use of in vitro
recombination techniques to insert a particular gene or
25 other DNA sequence into a vector molecule. In order to
successfully clone a desired gene, it is necessary to
employ methods for generating DNA fragments for joining
the fragments to vector molecules, for introducing the
composite DNA molecule into a host cell in which it can
30 replicate, and for selecting the clone having the target
gene from amongst the recipient host cells.

     By "cDNA" is meant complementary or copy DNA
produced from an RNA template by the action of RNA-
dependent DNA polymerase (reverse transcriptase). Thus a

"cDNA clone" means a duplex DNA sequence complementary to
an RNA mol cule of interest, carried in a cloning vector.

By "cDNA library" is meant a collection of
recombinant DNA molecules containing cDNA inserts which
5   comprise DNA copies of mRNA being expressed by the cell
at the time the cDNA library was made. Such a cDNA
library may be prepared by methods known to those of
skill, and described, for example, in Maniatis et al.,
Molecular Cloning: A Laboratory Manual, supra.
10 Generally, RNA is first isolated from the cells of an
organism from whose genome it is desired to clone a
particular gene. Preferred for the purpose of the
present invention are mammalian, and particularly human,
lymphocytic cell lines. A presently preferred vector for
15 this purpose is the vaccinia virus WR strain.

By "vector" is meant a DNA molecule, derived,
e.g., from a plasmid, bacteriophage, or mammalian or
insect virus, into which fragments of DNA may be inserted
or cloned. A vector will contain one or more unique
20 restriction sites and may be capable of autonomous
replication in a defined host or vehicle organism such
that the cloned sequence is reproducible. Thus, by "DNA
expression vector" is meant any autonomous element
capable of directing the synthesis of a recombinant
25 peptide. Such DNA expression vectors include bacterial
plasmids and phages and mammalian and insect plasmids and
viruses.

By "substantially pure" is meant a compound, e.g.,
a a protein, a polypeptide, or an antibody, that is
30 substantially free of the components that naturally
accompany it. Generally, a compound is substantially
pure when at least 60%, more preferably at least 75%, and
most preferably at least 90% of the total material in a
sample is the compound of interest. Purity can be
35 measured by any appropriate method, e.g., column

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chromatography, polyacrylamide gel electrophoresis, r
HPLC analysis. In the context of a nucl ic acid,
"substantially pure" means a nucleic acid sequence,
segment, or fragment that is free from the genes that
5   flank it in its naturally-occurring state (e.g., free
from the sequences that flank the nucleic acid in its
native genomic position). By "functional derivative" is
meant the "fragments," "variants," "analogues," or
"chemical derivatives" of a molecule. A "fragment" of a
10 molecule, such as any of the cDNA sequences of the
present invention, is meant to refer to any nucleotide
subset of the molecule. A "variant" of such molecule is
meant to refer to a naturally occurring molecule
substantially similar to either the entire molecule, or a
15 fragment thereof. An "analog" of a molecule is meant to
refer to a non-natural molecule substantially similar to
either the entire molecule or a fragment thereof. A
molecule is said to be "substantially similar" to another
molecule if the sequence of amino acids in both molecules
20 is substantially the same. Substantially similar amino
acid molecules will possess a similar biological
activity. Thus, provided that two molecules possess a
similar activity, they are considered variants as that
term is used herein even if one of the molecules contains
25 additional or fewer amino acid residues not found in the
other, or if the sequence of amino acid residues is not
identical. As used herein, a molecule is said to be a
"chemical derivative" of another molecule when it
contains additional chemical moieties not normally a part
30 of the molecule. Such moieties may improve the
molecule's solubility, absorption, biological half life,
etc. The moieties may alternatively decrease the
toxicity of the molecule, eliminate or attenuate any
undesirable side effect of the molecule, etc. Moieties
35 capable of mediating such effects are disclosed, for

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example, in <u>Remington's Pharmaceutical Sciences</u>, 16th
et., Mack Publishing Co., Easton, Penn. (1980).

Similarly, a "functional derivative" of a receptor
chimera of the present invention is meant to include
5   "fragments," "variants," or "analogues" of the gene,
which may be "substantially similar" in nucleotide
sequence, and which encode a molecule possessing similar
activity to, for example, a T cell, B cell, or Fc
receptor chimera.

10   Thus, as used herein, a T cell, B cells or Fc
receptor chimera protein is also meant to include any
functional derivative, fragments, variants, analogues, or
chemical derivatives which may be substantially similar
to the "wild-type" chimera and which possess similar
15 activity (i.e., most preferably, 90%, more preferably,
70%, preferably 40%, or at least 10% of the wild-type
receptor chimera's activity). The activity of a
functional chimeric receptor derivative includes specific
binding (with its extracellular portion) to a targeted
20 agent or cell and resultant destruction (directed by its
intracellular or transmembrane portion) of that agent or
cell; such activity may be tested, e.g., using any of the
assays described herein.

A DNA sequence encoding the T cell, B cell, or Fc
25 receptor chimera of the present invention, or its
functional derivatives, may be recombined with vector DNA
in accordance with conventional techniques, including
blunt-ended or staggered-ended termini for ligation,
restriction enzyme digestion to provide appropriate
30 termini, filling in of cohesive ends as appropriate,
alkaline phosphatase treatment to avoid undesirable
joining, and ligation with appropriate ligases.
Techniques for such manipulations are disclosed by
Maniatis, T., et al., <u>supra</u>, and are well known in the
35 art.

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A nucleic acid molecule, such as DNA, is said to
be "capable of expressing" a polypeptide if it contains
nucleotide sequences which contain transcriptional and
translational regulatory information and such sequences
5   are "operably linked" to nucleotide sequences which
encode the polypeptide. An operable linkage is a linkage
in which the regulatory DNA sequences and the DNA
sequence sought to be expressed are connected in such a
way as to permit gene expression. The precise nature of
10 the regulatory regions needed for gene expression may
vary from organism to organism, but shall in general
include a promoter region which, in prokaryotes, contains
both the promoter (which directs the initiation of RNA
transcription) as well as the DNA sequences which, when
15 transcribed into RNA, will signal the initiation of
protein synthesis. Such regions will normally include
those 5'-non-coding sequences involved with initiation of
transcription and translation, such as the TATA box,
capping sequence, CAAT sequence, and the like.
20   If desired, the non-coding region 3' to the gene
sequence coding for the protein may be obtained by the
above-described methods. This region may be retained for
its transcriptional termination regulatory sequences,
such as termination and polyadenylation. Thus, by
25 retaining the 3'-region naturally contiguous to the DNA
sequence coding for the protein, the transcriptional
termination signals may be provided. Where the
transcriptional termination signals are not
satisfactorily functional in the expression host cell,
30 then a 3' region functional in the host cell may be
substituted.
Two DNA sequences (such as a promoter region
sequence and a T cell receptor, a B cell receptor, or Fc
receptor chimera encoding sequence) are said to be
35 operably linked if the nature of the linkage between the

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two DNA sequences does not (1) result in the introduction
f a frame-shift mutation, (2) interfere with the ability
f the promoter region sequence to direct the
transcription of the receptor chimera gene sequence, or
5   (3) interfere with the ability of the receptor chimera
gene sequence to be transcribed by the promoter region
sequence. A promoter region would be operably linked to
a DNA sequence if the promoter were capable of effecting
transcription of that DNA sequence. Thus, to express the
10 protein, transcriptional and translational signals
recognized by an appropriate host are necessary.

     The present invention encompasses the expression
of a T cell receptor, B cell receptor, or Fc receptor
chimera protein (or a functional derivative thereof) in
15 either prokaryotic or eukaryotic cells, although
eukaryotic (and, particularly, human lymphocyte)
expression is preferred.

     Antibodies according to the present invention may
be prepared by any of a variety of methods. For example,
20 cells expressing the receptor chimera protein, or a
functional derivative thereof, can be administered to an
animal in order to induce the production of sera
containing polyclonal antibodies that are capable of
binding the chimera.

25   In a preferred method, antibodies according to the
present invention are monoclonal antibodies. Such
monoclonal antibodies can be prepared using hybridoma
technology (Kohler et al., Nature 256:495 (1975); Kohler
et al., Eur. J. Immunol. 6:511 (1976); Kohler et al.,
30 Eur. J. Immunol. 6:292 (1976); Hammerling et al., In:
Monoclonal Antibodies and T-Cell Hybridomas, Elsevier,
N.Y., pp. 563-684 (1981)). In general, such procedures
involve immunizing an animal with the T cell receptor, B
cell receptor, or Fc receptor chimera antigen. The
35 splenocytes of such animals are extracted and fused with

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a suitable myeloma cell line. Any suitable myeloma cell
line may be employ d in accordance with the present
invention. After fusion, the resulting hybridoma cells
are selectively maintained in HAT medium, and then cloned
5 by limiting dilution as described by Wands, J.R., et al.
(Gastroenterology 80:225-232 (1981). The hybridoma cells
obtained through such a selection are then assayed to
identify clones which secrete antibodies capable of
binding the chimera.

10   Antibodies according to the present invention also
may be polyclonal, or, preferably, region specific
polyclonal antibodies.

Antibodies against the T cell receptor, B cell
receptor, or Fc receptor chimera according to the present
15 invention may be used to monitor the amount of chimeric
receptor (or chimeric receptor-bearing cells) in a
patient. Such antibodies are well suited for use in
standard immunodiagnostic assay known in the art,
including such immunometric or "sandwich" assays as the
20 forward sandwich, reverse sandwich, and simultaneous
sandwich assays. The antibodies may be used in any
number of combinations as may be determined by those of
skill without undue experimentation to effect
immunoassays of acceptable specificity, sensitivity, and
25 accuracy.

Standard reference works setting forth general
principles of immunology include Roitt, I., Essential
Immunology, Sixth Ed., Blackwell Scientific Publications,
Publisher, Oxford (1988); Kimball, J. W., Introduction to
30 Immunology, Second Ed., Macmillan Publishing Co.,
Publisher, New York (1986); Roitt, I., et al.,
Immunology, Gower Medical Publishing Ltd., Publisher,
London, (1985); Campbell, A. , "Monoclonal Antibody
Technology," in, Burdon, R., et al., eds., Laboratory
35 Techniques in Biochemistry and Molecular Biology, Volume

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13, Elsevier, Publisher, Amsterdam (1984); Klein, J.,
Immunology: The Science of Self-Nonself Discrimination,
John Wiley & Sons, Publisher, New York (1982); and
Kennett, R., et al., eds., _Monoclonal Antibodies,_
5 _Hybridoma: A New Dimension In Biological Analyses_,
Plenum Press, Publisher, New York (1980).

By "detecting" it is intended to include
determining the presence or absence of a substance or
quantifying the amount of a substance. The term thus
10 refers to the use of the materials, compositions, and
methods of the present invention for qualitative and
quantitative determinations.

The isolation of other hybridomas secreting
monoclonal antibodies of the same specificity as those
15 described herein can be accomplished by the technique of
anti-idiotypic screening (Potocmjak, et al., _Science_
215:1637 (1982)). Briefly, an anti-idiotypic antibody is
an antibody which recognizes unique determinants present
on the antibody produced by the clone of interest. The
20 anti-idiotypic antibody is prepared by immunizing an
animal of the same strain used as the source of the
monoclonal antibody with the monoclonal antibody of
interest. The immunized animal will recognize and
respond to the idiotypic determinants of the immunizing
25 antibody by producing antibody to these idiotypic
determinants (anti-idiotypic antibody).

For replication, the hybrid cells may be
cultivated both _in vitro_ and _in vivo_. High _in vivo_
production makes this the presently preferred method of
30 culture. Briefly, cells from the individual hybrid
strains are injected intraperitoneally into pristane-
primed BALB/c mice to produce ascites fluid containing
high concentrations of the desired monoclonal antibodies.
Monoclonal antibodies of isotype IgM or IgG may be
35 purified from cultured supernatants using column

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chromatography methods well known to those of skill in the art.

Antibodies according to the present invention are particularly suited for use in immunoassays wherein they
5 may be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways.

There are many different labels and methods of labeling known in the art. Examples of the types of
10 labels which can be used in the present invention include, but are not limited to, enzymes, radioisotopes, fluorescent compounds, chemiluminescent compounds, bioluminescent compounds and metal chelates. Those of ordinary skill in the art will know of other suitable
15 labels for binding to antibodies, or will be able to ascertain the same by the use of routine experimentation. Furthermore, the binding of these labels to antibodies can be accomplished using standard techniques commonly known to those of ordinary skill in the art.
20   One of the ways in which antibodies according to the present invention can be detectably labeled is by linking the antibody to an enzyme. This enzyme, in turn, when later exposed to its substrate, will react with the substrate in such a manner as to produce a chemical
25 moiety which can be detected as, for example, by spectrophotometric or fluorometric means. Examples of enzymes which can be used to detectably label antibodies include malate dehydrogenase, staphylococcal nuclease, delta-V-steroid isomerase, yeast alcohol dehydrogenase,
30 alpha-glycerophosphate dehydrogenase, triose phosphate isomerase, biotinavidin peroxidase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-VI-phosphate dehydrogenase, glucoamylase and
35 acetylcholine esterase.

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The presenc of detectably labeled antibodies also can be detected by labeling the antibodies with a radioactive isotope which then can be determined by such means as the use of a gamma counter or a scintillation counter. Isotopes which are particularly useful for the purpose of the present invention are ^{3}H, ^{125}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{36}Cl, ^{57}Co, ^{58}Co, ^{59}Fe and ^{75}Se.

It is also possible to detect the binding of detectably labeled antibodies by labeling the antibodies with a fluorescent compound. When a fluorescently labeled antibody is exposed to light of the proper wavelength, its presence then can be detected due to the fluorescence of the dye. Among the most commonly used fluorescent labeling compounds are fluorescein, isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, o-phthaldehyde and fluorescamine.

The antibodies of the invention also can be detectably labeled using fluorescent emitting metals such as ^{152}Eu, or others of the lanthanide series. These metals can be attached to the antibody molecule using such metal chelating groups as diethyl-enteriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Antibodies also can be detectably labeled by coupling them to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of the chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminal, isoluminol, theromatic acridinium ester, imidazole, acridinium salts, oxalate ester, and dioxetane.

Likewise, a bioluminescent compound may be used to label the antibodies according to the present invention. Bioluminescence is a type of chemiluminescence found in

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biological systems in which a catalytic protein increases
the fficiency of the chemiluminescent reaction. The
presence of a bioluminescent antibody is determined by
detecting the presence of luminescence. Important
5 bioluminescent compounds for purposes of labeling include
luciferin, luciferase aequorin.

The antibodies and substantially purified antigen
of the present invention are ideally suited for the
preparation of a kit. Such a kit may comprise a carrier
10 means being compartmentalized to receive in close
confinement therewith one or more container means such as
vials, tubes and the like, each of said container means
comprising the separate elements of the assay to be used.

The types of assays which can be incorporated in
15 kit form are many, and include, for example, competitive
and non-competitive assays. Typical examples of assays
which can utilize the antibodies of the invention are
radioimmunoassays (RIA), enzyme immunoassays (EIA),
enzyme-linked immunosorbent assays (ELISA), and
20 immunometric, or sandwich, immunoassays.

By the term "immunometric assay" or "sandwich
immunoassay," it is meant to include simultaneous
sandwich, forward sandwich and reverse sandwich
immunoassays. These terms are well understood by those
25 skilled in the art. Those of skill will also appreciate
that antibodies according to the present invention will
be useful in other variations and forms of assays which
are presently known or which may be developed in the
future. These are intended to be included within the
30 scope of the present invention.

In the preferred mode for performing the assays it
is important that certain "blockers" be present in the
incubation medium (usually added with the labeled soluble
antibody). The "blockers" are added to assure that non-
35 specific proteins, protease, or human antibodies to mouse

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immunoglobulins present in the experimental sample do not
cr ss-link or destroy the antibodies on the solid phase
support, or the radiolabeled indicator antibody, to yield
false positive or false negative results. The selection
5 of "blockers" therefore adds substantially to the
specificity of the assays described in the present
invention.

It has been found that a number of nonrelevant
(i.e., nonspecific) antibodies of the same class or
10 subclass (isotype) as those used in the assays (e.g.,
IgG_1, IgG_{2a}, IgM, etc.) can be used as "blockers." The
concentration of the "blockers" (normally 1-100 $\mu g/\mu l$) is
important, in order to maintain the proper sensitivity
yet inhibit any unwanted interference by mutually
15 occurring cross reactive proteins in human serum. In
addition, the buffer system containing the "blockers"
needs to be optimized. Preferred buffers are those based
on weak organic acids, such as imidazole, HEPPS, MOPS,
TES, ADA, ACES, HEPES, PIPES, TRIS, and the like, at
20 physiological pH ranges. Somewhat less preferred buffers
are inorganic buffers such as phosphate, borate or
carbonate. Finally, known protease inhibitors should be
added (normally at 0.01-10 $\mu g/ml$) to the buffer which
contains the "blockers."
25   There are many solid phase immunoadsorbents which
have been employed and which can be used in the present
invention. Well known immunoadsorbents include glass,
polystyrene, polypropylene, dextran, nylon and other
materials, in the form of tubes, beads, and microtiter
30 plates formed from or coated with such materials, and the
like. The immobilized antibodies can be either
covalently or physically bound to the solid phase
immunoadsorbent, by techniques such as covalent bonding
via an amide or ester linkage, or by absorption. Those
35 skilled in the art will know many other suitable solid

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phase immunoadsorbents and methods for immobilizing
antibodies thereon, or will be able to ascertain such,
using no more than routine experimentation.

For _in vivo_, _in vitro_, or _in situ_ diagnosis,
5 labels such as radionuclides may be bound to antibodies
according to the present invention either directly or by
using an intermediary functional group. An intermediary
group which is often used to bind radioisotopes which
exist as metallic cations to antibodies is
10 diethylenetriaminepentaacetic acid (DTPA). Typical
examples of metallic cations which are bound in this
manner are: ^{99m}Tc, ^{123}I, ^{111}IN, ^{131}I, ^{97}Ru, ^{67}Cu, ^{67}Ga and
^{68}Ga. The antibodies of the invention can also be labeled
with non-radioactive isotopes for purposes of diagnosis.
15 Elements which are particularly useful in this manner are
^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Cr and ^{56}Fe.

The antigen of the invention may be isolated in
substantially pure form employing antibodies according to
the present invention. Thus, an embodiment of the
20 present invention provides for substantially pure T cell
receptor, B cell receptor, or Fc receptor chimera, said
antigen characterized in that it is recognized by and
binds to antibodies according to the present invention.
In another embodiment, the present invention provides a
25 method of isolating or purifying the receptor chimeric
antigen, by forming a complex of said antigen with one or
more antibodies directed against the receptor chimera.

The substantially pure T cell receptor, B cell
receptor, or Fc receptor chimera antigens of the present
30 invention may in turn be used to detect or measure
antibody to the chimera in a sample, such as serum or
urine. Thus, one embodiment of the present invention
comprises a method of detecting the presence or amount of
antibody to receptor chimera antigen in a sample,
35 comprising contacting a sample containing an antibody to

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the chimeric antigen with detectably labeled receptor
chimera, and detecting said label. It will be
appreciated that immunoreactive fractions and
immunoreactive analogues of the chimera also may be used.

5 By the term "immunoreactive fraction" is intended any
portion of the chimeric antigen which demonstrates an
equivalent immune response to an antibody directed
against the receptor chimera. By the term
"immunoreactive analogue" is intended a protein which

10 differs from the receptor chimera protein by one or more
animo acids, but which demonstrates an equivalent
immunoresponse to an antibody of the invention.

By "specifically recognizes and binds" is meant an
antibody which recognizes and binds a chimeric receptor

15 polypeptide but which does not substantially recognize
and bind other molecules in a sample, e.g., in a
biological sample, which includes the receptor
polypeptide.

By "autoimmune-generated cell" is meant cells

20 producing antibodies that react with host tissue or
immune effector T cells that are autoreactive; such cells
include antibodies against acetylcholine receptors
(leading, e.g., to myasthenia gravis) or anti-DNA, anti-
erythrocyte, and anti-placelet autoantibodies (leading,

25 e.g., to lupus erythematosus).

By "therapeutic cell" is meant a cell which has
been transformed by a chimera of the invention so that it
is capable of recognizing and destroying a specific
infective agent, a cell infected by a specific agent, a

30 tumor or cancerous cell, or an autoimmune-generated cell;
preferably such therapeutic cells are cells of the
hematopoietic system.

By "extracellular" is meant having at least a
portion of the molecule exposed at the cell surface. By

35 "intracellular" is meant having at least a portion of the

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molecule exposed to the therapeutic cell's cytoplasm. By "transmembrane" is meant having at least a portion of the molecule spanning the plasma membrane. An "extracellular portion", an "intracellular portion" and a "transmembrane
5 portion", as used herein, may include flanking amino acid sequences which extend into adjoining cellular compartments.

By "oligomerize" is meant to complex with other proteins to form dimers, trimers, tetramers, or other
10 higher order oligomers. Such oligomers may be homo-oligomers or hetero-oligomers. An "oligomerizing portion" is that region of a molecule which directs complex (i.e., oligomer) formation.

By "cytolytic" is meant to be capable of
15 destroying a cell (e.g., a cell infected with a pathogen, a tumor or cancerous cell, or an autoimmune-generated) cell or to be capable of destroying an infective agent (e.g., a virus).

By "immunodeficiency virus" is meant a retrovirus
20 that, in wild-type form, is capable of infecting T4 cells of a primate host and possesses a viral morphogenesis and morphology characteristic of the lentivirus subfamily. The term includes, without limitation, all variants of HIV and SIV, including HIV-1, HIV-2, SIVmac, SIVagm,
25 SIVmnd, SIVsmm, SIVman, SIVmand, and SIVcpz.

By "MHC-independent" is meant that the cellular cytolytic response does not require the presence of an MHC class II antigen on the surface of the targeted cell.

By a "functional cytolytic signal-transducing
30 derivative" is meant a functional derivative (as defined above) which is capable of directing at least 10%, preferably 40%, more preferably 70%, or most preferably at least 90% of the biological activity of the wild type molecule. As used herein, a "functional cytolytic
35 signal-transducing derivative" may act by directly

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signaling the th rapeutic c 11 to destroy a receptor-
bound agent or cell (e.g., in th case of an
intracellular chimeric receptor portion) or may act
indirectly by promoting oligomerization with cytolytic
5 signal transducing proteins of the therapeutic cell
(e.g., in the case of a transmembrane domain). Such
derivatives may be tested for efficacy, e.g., using the
in vitro assays described herein.

       By a "functional HIV envelope-binding derivative"
10 is meant a functional derivative (as defined above) which
is capable of binding any HIV envelope protein.
Functional derivatives may be identified using, e.g., the
in vitro assays described herein.

THERAPEUTIC ADMINISTRATION
15   The transformed cells of the present invention may
be used for the therapy of a number of diseases. Current
methods of administering such transformed cells involve
adoptive immunotherapy or cell-transfer therapy. These
methods allow the return of the transformed immune-system
20 cells to the bloodstream. Rosenberg, S.A., Scientific
American, 62 (May 1990); Rosenberg et al., The New
England Journal of Medicine, 323(9):570 (1990).

       The pharmaceutical compositions of the invention
may be administered to any animal which may experience
25 the beneficial effects of the compounds of the invention.
Foremost among such animals are humans, although the
invention is not intended to be so limited.

                   Detailed Description
The drawings will first be described.

30   Brief Description of the Drawings
       FIG. 1 Characterization of CD4 chimeras. Fig. 1A
presents the amino acid sequence about the site of fusion

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betw en CD4 (residues 1-369) and the different r ceptor chains. The underlined sequence shows the position of the amino acids encoded within the BamHI site used for fusion construction. The beginning of the transmembrane
5 domain is marked with a vertical bar. The η sequence is identical to the ζ sequence at the amino terminus, but diverges at the carboxyl terminus (Jin et al., <u>Proc. Natl. Acad. Sci. USA</u>, <u>87</u>:3319-3323 (1990)). Fig. 1B presents flow cytometric analysis of surface expression
10 of CD4, CD4:ζ, CD4:γ and CD4:η in CV1 cells. Cells were infected with virus expressing CD4 chimeras or $CD16_{PI}$, incubated for 9 hours at 37°C, and stained with phycoerythrin-conjugated anti-CD4 MAb Leu3A. Fig. 1C presents immunoprecipitation of labeled CD4:ζ, CD4:γ, or
15 native CD4 expressed in CV1 cells. Lanes were run with reducing (R) or without reducing (NR) agent. Molecular mass standards in kD are shown at left.

FIG. 2 Surface expression of $CD16_{TM}$ following coinfection of $CD16_{TM}$ alone (dense dots), or coinfected
20 with virus expressing CD4:γ (dashes) or CD4:ζ (solid line). Sparse dots, cells infected with CD4:ζ alone, stained with 3G8 (Fleit et al., <u>Proc. Natl. Acad. Sci. USA</u>, <u>79</u>:3275-3279 (1982)) (anti-CD16 MAb).

FIG. 3 Mutant CD4:ζ chimeric receptors lacking ζ
25 Asp-15 do not support the coexpression of $CD16_{TM}$. Fig. 3A is an autoradiogram of immunoprecipitated mutant chimeras electrophoresed either with reduction (R) or without reduction (NR). Fig. 3B details surface expression of $CD16_{TM}$ following coinfection by viruses expressing $CD16_{TM}$
30 and the following ζ chimeras: CD4:ζ (thick line), CD4:ζ C11G (solid line); CD4:ζ (dashed line); CD4:ζ C11G/D15G (dense dots); no coinfection ($CD16_{TM}$ alone, sparse dots). Cells were incubated with anti-CD16 MAb 3G8 and phycoerythrin- conjugated Fab'$_2$ goat antibodies to mouse
35 IgG. The level of expression of the ζ chimeras was

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essentially identical for the differ nt mutants analyzed, and coinfection of cells with viruses expressing CD16$_{TM}$ and ς chimeras did not appreciably alter surface expression of the chimeras (data not shown).

5   **FIG. 4** Increased intracellular free calcium ion follows crosslinking of mutant ς chimeras in a T cell line. Jurkat E6 cells (Weiss et al., *J. Immunol.*, 133:123-128 (1984)) were infected with recombinant vaccinia viruses and analyzed by flow cytometry. The

10 results shown are for the gated CD4$^+$ population, so that only cells expressing the relevant chimeric protein are analyzed. The mean ratio of violet to blue Indo-1 fluorescence reflects the intracellular free calcium concentration in the population as a whole and the

15 percentage of responding cells reflects the fraction of cells which exceed a predetermined threshold ratio (set so that 10% of untreated cells are positive). Fig. 4A and Fig. 4B show Jurkat cells expressing CD4:ς (solid line) or CD16:ς (dashed line) which were exposed to anti-

20 CD4 MAb Leu3a (phycoerythrin conjugate), followed by crosslinking with goat antibody to mouse IgG. The dotted line shows the response of uninfected cells to anti-CD3 MAb OKT3. Figs. 4C and 4D show Jurkat cells expressing CD4:ςD15G (solid line); CD4:ςC11G/D15G (dashes); or

25 CD4:ςC11G (dots) which were treated and analyzed as in Figs. 4A and 4B.

          FIG. 5 CD4:ς, CD4:η, and CD4:γ receptors allow cytolytic T lymphocytes (CTL) to kill targets expressing HIV-1 gp120/41. Fig. 5A: solid circles, CTL expressing

30 CD4:ς incubated with HeLa cells expressing gp120/41; open circles, CTL expressing CD4:ς incubated with uninfected HeLa cells; solid squares, uninfected CTL incubated with HeLa cells expressing gp120/41; open squares, uninfected CTL incubated with uninfected HeLa cells. Fig. 5B:

35 solid circles, CTL expressing CD4:η incubated with HeLa

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cells xpressing gp120/41; open circles, CTL expressing
CD4:γ incubated with HeLa cells expressing gp120/41; open
squares, CTL expressing the C11G/D15G double mutant CD4:ζ
chimera incubated with HeLa cells expressing gp120/41.

5   Fig. 5C: Flow cytometric analysis of CD4 expression by
the CTL used in Fig. 5B. To correct the target to
effector ratios the percent of cells expressing CD4
chimera was determined by subtracting the scaled negative
(uninfected) population by histogram superposition; for
10 comparative purposes in this figure the uninfected cells
were assigned an arbitrary threshold which gives roughly
the same fraction positive for the other cell populations
as would histogram subtraction.

    FIG. 6 Specificity of the CD4-directed cytolysis.
15 Fig. 6A: solid circles, CTL expressing CD4:ζ incubated
with HeLa cells expressing CD16$_{PI}$; open circles, CTL
expressing CD4 incubated with HeLa cells expressing
gp120; solid squares, CTL expressing CD16:ζ incubated
with HeLa cells expressing gp120/41; open squares, CTL
20 expressing CD16$_{PI}$ incubated with HeLa cells expressing
gp120/41. Fig. 6B: solid circles, CTL expressing CD4:ζ
incubated with Raji (MHC class II$^+$) cells; open circles,
uninfected CTL cells incubated with RJ2.2.5 (MHC class
II$^-$ Raji mutant) cells; solid squares, uninfected CTL
25 incubated with Raji (MHC class II$^+$) cells; open squares,
CTL expressing CD4:ζ incubated with RJ2.2.5 (MHC class
II$^-$) cells. The ordinate scale is expanded.

    FIG. 7 Characterization of the CD16:ζ chimeric
receptor. Fig. 7A is a schematic diagram of the CD16:ζ
30 fusion protein. The extracellular portion of the
phosphatidylinositol-linked form of monomeric CD16 was
joined to dimeric ζ just external to the transmembrane
domain. The protein sequence at the fusion junction is
shown at the bottom. Fig. 7B shows a flow cytometric
35 analysis of calcium mobilization following crosslinking

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of th CD16:ʃ chimera in either a TCR positive or TCR
positive r TCR negative cell line. Th mean ratio of
violet to blue fluorescence (a measure of relative
calcium ion concentration) among cell populations treated
5   with antibodies at time 0 is shown. Solid squares, the
response of Jurkat cells to anti-CD3 MAb OKT3; solid
triangles, the response of CD16:ʃ to anti-CD16 MAb 3G8
crosslinking in the REX33A TCR⁻ mutant; open squares, the
response to CD16:ʃ crosslinking in the Jurkat TCR⁻ mutant
10   line JRT3.T3.5; open triangles, the response to CD16:ʃ
crosslinking in Jurkat cells; crosses, the response to
nonchimeric CD16 in Jurkat cells; and dots, the response
to nonchimeric CD16 in the REX33A TCR⁻ cell line.

FIG. 8 Deletion analysis of cytolytic potential.
15   Fig. 8A shows the locations of the ʃ deletion endpoints.
Here as elsewhere mutations in ʃ are represented by the
original residue-location-mutant residue convention, so
that D66*, for example, denotes replacement of Asp-66 by
a termination codon. Fig. 8B shows cytolysis assay
20   results of undeleted CD16:ʃ and salient ʃ deletions.
Hybridoma cells expressing surface antibody to CD16 were
loaded with ^{51}Cr and incubated with increasing numbers of
human cytolytic lymphocytes (CTL) infected with vaccinia
recombinants expressing CD16:ʃ chimeras. The percent of
25   ^{51}Cr released is plotted as a function of the effector
(CTL) to target (hybridoma) cell ratio (e/t). Solid
circles, cytolysis mediated by cells expressing CD16:ʃ
(mfi 18.7); solid squares, cytolysis mediated by cells
expressing CD16:ʃ Asp66* (mfi 940.2); open squares,
30   cytolysis mediated by cells expressing CD16:ʃGlu60* (mfi
16.0); open circles, cytolysis mediated by cells
expressing CD16:ʃTyr51* (mfi 17.4); solid triangles,
cytolysis mediated by cells expressing CD16:ʃPhe34* (mfi
17.8); and open triangles, cytolysis mediated by cells
35   expressing nonchimeric CD16 (mfi 591). Although in this

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experiment th expr ssion of CD16:ʕAsp66* was not matched
to that of the other fusion proteins, cytolysis by cells
expressing CD16:ʕ at equivalent levels in the same
experiment gave results essentially identical to those
5   shown by cells expressing CD16:ʕAsp66* (not shown).

    FIG. 9 Elimination of the potential for
transmembrane interactions reveals a short ʕ segment
capable of mediating cytolysis. Fig. 9A is a schematic
diagram of the monomeric bipartite and tripartite
10   chimeras. At the top is the CD16:ʕ construct truncated
at residue 65 and lacking transmembrane Cys and Asp
residues. Below are the CD16:CD5:ʕ and CD16:CD7:ʕ
constructs and related controls. The peptide sequences
of the intracellular domains are shown below. Fig. 9B
15   shows the cytolytic activity of monomeric chimera
deletion mutants. The cytolytic activity of cells
expressing CD16:ʕ (solid circles; mfi 495) was compared
to that of cells expressing CD16:ʕAsp66* (solid squares;
mfi 527) or the mutants CD16:ʕCys11Gly/Asp15Gly/Asp66*,
20   (open squares; mfi 338) and
CD16:ʕCys11Gly/Asp15Gly/Glu60* (filled triangles; mfi
259). Fig. 9C shows the cytolytic activity mediated by
tripartite fusion proteins. Solid triangles,
CD16:ʕAsp66*; open squares, CD16:5:ʕ(48-65); solid
25   squares CD16:7:ʕ(48-65); open triangles, CD16:7:ʕ(48-
59); open circles, CD16:5; solid circles, CD16:7. Fig.
9D shows calcium mobilization by mutant and tripartite
chimeras in the TCR negative Jurkat JRT3.T3.5 mutant cell
line. Open circles, response of cells expressing dimeric
30   CD16:ʕAsp66*; solid squares, response of cells expressing
CD16:ʕCys11Gly/Asp15Gly/Asp66*; open squares, response of
cells expressing CD16:ʕCys11Gly/Asp15Gly/Glu60*; solid
triangles, response of cells expressing CD16:7:ʕ(48-65);
and open triangles, response of cells expressing
35   CD16:ʕ(48-59).

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FIG. 10 Contribution of individual amino acids to
the activity of th 18 residue cytolytic signal-
transducing motif. Figs. 10A and 10B show cytolytic
activity and Fig. 10C shows calcium ion mobilization
5   mediated by chimeras bearing point mutations near the
carboxyl terminal tyrosine (Y62). Figs. 10A and 10B
represent data collected on cells expressing low and high
amounts, respectively, of the CD16:ζ fusion proteins.
Identical symbols are used for the calcuim mobilization
10   and cytolysis assays, and are shown in one letter code at
right. Solid circles, cells expressing CD16:ζ (mfi in A,
21; B, 376); solid squares, cells expressing CD16:7:ζ(48-
65) (mfi A, 31; B, 82); open squares, CD16:7:ζ(48-
65)Glu60Gln (mfi A, 33; B, 92), crosses, CD16:7:ζ(48-
15   65)Asp63Asn (mfi A, 30; B, 74); solid triangles,
CD16:7:ζ(48-65)Tyr62Phe (mfi A, 24; B, 88); open circles,
CD16:7:ζ(48-65)Glu61Gln (mfi A, 20; B, 62); and open
triangles, CD16:7:ζ(48-65)Tyr62Ser (mfi B, 64). Figs.
10D and 10E show cytolytic activity and Fig. 10F shows
20   calcium ion mobilization by chimeras bearing point
mutations near the amino terminal tyrosine (Y51).
Identical symbols are sued for the calcium mobilization
and cytolysis assays and are shown at right. Solid
circles, cells expressing CD16:ζ (mfi in D, 21.2; in E,
25   672); solid squares, cells expressing CD16:7:ζ(48-65)
(mfi D, 31.3; E, 179); solid triangles, CD16:7:ζ(48-
65)Asn48Ser (mfi D, 22.4; E, 209); open squares,
CD16:7:ζ(48-65)Leu50Ser (mfi D, 25.0; E, 142); and open
triangles, CD16:7:ζ(48-65)Tyr51Phe (mfi D, 32.3; E, 294).
30   FIG. 11 Alignment of internal repeats of ζ and
comparison of their ability to support cytolysis. Fig.
11A is a schematic diagram of chimeras formed by dividing
the ζ intracellular domain into thirds and appending them
to the transmembrane domain of a CD16:7 chimera. The
35   sequences of the intracellular domains are shown below,

with shared residu s boxed, and related residues denoted
by asterisks. Fig. 11B shows the cytolytic potency f
the three ς subdomains. Solid circles, cells expressing
CD16:ς (mfi 476); solid squares, CD16:7:ς(33-65) (mfi

5 68); open squares, CD16:7:ς(71-104) (mfi 114); and solid
triangles, CD16:7:ς(104-138) (mfi 104).

FIG. 12 is a schematic diagram of the CD16:FcRγII
chimeras.

FIG. 13 Calcium mobilization following

10 crosslinking of CD4:FcRγII and CD16:FcRγII chimeras.
Fig. 13A shows the ratio of violet to blue fluorescence
emitted by cells loaded with the calcium sensitive
fluorophore Indo-1 shown as a function of time following
crosslinking of the CD16 extracellular domain with

15 antibodies. Fig. 13B shows a similar analysis of the
increase in ratio of violet to blue fluorescence of cells
bearing CD4:FcRγII chimeras, following crosslinking with
antibodies.

FIG. 14 Cytolysis assay of CD4:FcRγII and

20 CD16:FcRγII chimeras. Fig. 14A shows the percent of [51]Cr
released from anti-CD16 hybridoma (target) cells when the
cells are exposed to increasing numbers of cytotoxic T
lymphocytes expressing CD16:FcRγII chimeras (effector
cells). Fig. 14B shows a similar analysis of

25 cytotoxicity mediated by CD4:FcRγII chimeras against
target cells expressing HIV envelope glycoproteins.

FIG. 15 Identification of residues in the FcRγII A
tail which are important for cytolysis. Fig. 15A is a
schematic diagram of the deletion constructs. Figs. 15B

30 and 15C shows calcium mobilization and cytolysis by
carboxyl-terminal deletion variants of CD16:FcRγII A.
Figs. 15D and 15E show calcium mobilization and cytolysis
by tripartite chimeras bearing progressively less of the
amino terminus of the intracellular tail of CD16:FcRγII

35 A.

FIG. 16 (SEQ ID NO: 24) shows the amino acid
s quence of th CD3 delta receptor protein; the boxed
sequence represents a preferred cytolytic signal
transducing portion.

5   FIG. 17 (SEQ ID NO: 25) shows the amino acid
sequence of the T3 gamma receptor protein; the boxed
sequence represents a preferred cytolytic signal
transducing portion.

FIG. 18 (SEQ ID NO: 26) shows the amino acid
10 sequence of the mb1 receptor protein; the boxed sequence
represents a preferred cytolytic signal transducing
portion.

FIG. 19 (SEQ ID NO: 27) shows the amino acid
sequence of the B29 receptor protein; the boxed sequence
15 represents a preferred cytolytic signal transducing
portion.

EXAMPLE I

Construction of Human IgG1:Receptor Chimeras

Human IgG1 heavy chain sequences were prepared by
20 joining sequences in the C_H3 domain to a cDNA fragment
derived from the 3' end of the transmembrane form of the
antibody mRNA. The 3' end fragment was obtained by
polymerase chain reaction using a tonsil cDNA library as
substrate, and oligonucleotides having the sequences:
25   CGC GGG GTG ACC GTG CCC TCC AGC AGC TTG GGC (SEQ
ID NO: 7) and
          CGC GGG GAT CCG TCG TCC AGA GCC CGT CCA GCT CCC
CGT CCT GGG CCT CA (SEQ ID NO: 8),
corresponding to the 5' and 3' ends of the desired DNA
30 fragments respectively. The 5' oligo is complementary to
a site in the C_H1 domain of human IgG1, and the 3' oligo
is complementary to a site just 5' of the sequences
encoding the membrane spanning domain. The PCR product
was digested with BstXI and BamHI and ligated between

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BstXI and BamHI sites of a semisynthetic IgG1 antibody
g ne bearing variable and constant regions. Foll wing
the insertion of the BstXI to BamHI fragment, the
amplified portions of the construct were replaced up to
5 the SmaI site in C_H3 by restriction fragment interchange,
so that only the portion between the SmaI site and the 3'
oligo was derived from the PCR reaction.

To create a human IgG1:ʓ chimeric receptor, the
heavy chain gene ending in a BamHI site was joined to the
10 BamHI site of the ʓ chimera described below, so that the
antibody sequences formed the extracellular portion.
Flow cytometry of COS cells transfected with a plasmid
encoding the chimera showed high level expression of
antibody determinants when an expression plasmid encoding
15 a light chain cDNA was cotransfected, and modest
expression of antibody determinants when the light chain
expression plasmid was absent.

Similar chimeras including human IgG1 fused to η
or γ (see below), or any signal-transducing portion of a
20 T cell receptor or Fc receptor protein may be constructed
generally as described above using standard techniques of
molecular biology.

To create a single transcription unit which would
allow both heavy and light chains to be expressed from a
25 single promoter, a plasmid encoding a bicistronic mRNA
was created from heavy and light chain coding sequences,
and the 5' untranslated portion of the mRNA encoding the
78kD glucose regulated protein, otherwise known as grp78,
or BiP. grp78 sequences were obtained by PCR of human
30 genomic DNA using primers having the sequences:

CGC GGG CGG CCG CGA CGC CGG CCA AGA CAG CAC (SEQ
ID NO: 9) and

CGC GTT GAC GAG CAG CCA GTT GGG CAG CAG CAG (SEQ
ID NO: 10)

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at the 5' and 3' ends respectively. Polymerase chain
reactions with th s oligos were performed in the
presence of 10% dimethyl sulfoxide. The fragment
obtained by PCR was digested with NotI and HincII and
5   inserted between NotI and HpaI sites downstream from
human IgG1 coding sequences. Sequences encoding a human
IgG kappa light chain cDNA were then inserted downstream
from the grp78 leader, using the HincII site and another
site in the vector. The expression plasmid resulting
10   from these manipulations consisted of the semisynthetic
heavy chain gene, followed by the grp78 leader sequences,
followed by the kappa light chain cDNA sequences,
followed by polyadenylation signals derived from an SV40
DNA fragment. Transfection of COS cells with the
15   expression plasmid gave markedly improved expression of
heavy chain determinants, compared to transfection of
plasmid encoding heavy chain determinants alone.

          To create a bicistronic gene comprising a heavy
chain/receptor chimera and a light chain, the upstream
20   heavy chain sequences can be replaced by any chimeric
heavy chain/ receptor gene described herein.

                         EXAMPLE II
          Construction of CD4 Receptor Chimeras
                  Human ζ (Weissman et al., Proc. Natl. Acad. Sci.
25   USA, 85:9709-9713 (1988b)) and γ (Küster et al., J. Biol.
Chem., 265:6448-6452 (1990)) cDNAs were isolated by
polymerase chain reaction from libraries prepared from
the HPB-ALL tumor cell line (Aruffo et al., Proc. Natl.
Acad. Sci. USA, 84:8573-8577 (1987b)) and from human
30   natural killer cells, while η cDNA (Jin et al., Proc.
Natl. Acad. Sci. USA, 87:3319-3323 (1990)) was isolated
from a murine thymocyte library. ζ, η and γ cDNAs were
joined to the extracellular domain of an engineered form
of CD4 possessing a BamHI site just upstream of the

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membrane spanning domain (Aruffo et al., <u>Proc. Natl.</u>
<u>Acad. Sci. USA</u>, <u>84</u>:8573-8577 (1987b); Zettlmeissl t al.,
<u>DNA Cell Biol.</u>, <u>9</u>347-353 (1990)) which was joined to the
BamHI site naturally present in the ς and η cDNAs at a

5   similar location a few residues upstream of the membrane
spanning domain (SEQ ID NOS: 1, 3, 4 and 6). To form the
fusion protein with γ a BamHI site was engineered into
the sequence at the same approximate location (Fig. 1;
SEQ ID NO: 2 and 5). The gene fusions were introduced

10   into a vaccinia virus expression plasmid bearing the <u>E.</u>
<u>coli</u> <u>gpt</u> gene as a selectable marker (M. Amiot and B.S.,
unpublished), and inserted into the genome of the
vaccinia WR strain by homologous recombination and
selection for growth in mycophenolic acid (Falkner et

15   al., <u>J. Virol.</u>, 62:1849-1854 (1988); Boyle et al., <u>Gene</u>,
<u>65</u>:123-128 (1988)). Flow cytometric analysis showed that
the vaccinia recombinants direct the abundant production
of CD4:ς and CD4:γ fusion proteins at the cell surface,
whereas the expression of CD4:η is substantially weaker

20   (Fig. 1). The latter finding is consistent with a recent
report that transfection of an η cDNA expression plasmid
into a murine hybridoma cell line gave substantially less
expression than transfection of a comparable ς expression
plasmid (Clayton et al., <u>J. Exp. Med.</u>, <u>172</u>:1243-1253

25   (1990)). Immunoprecipitation of cells infected with the
vaccinia recombinants revealed that the fusion proteins
form covalent dimers, unlike the naturally occurring CD4
antigen (Fig. 1). The molecular masses of the monomeric
CD4:ς and CD4:γ fusion proteins and native CD4 were found

30   to be 63, 55 and 53 kD respectively. The larger masses
of the fusion proteins are approximately consistent with
the greater length of the intracellular portion, which
exceeds that of native CD4 by 75 (CD4:ς) or 5 (CD4:γ)
residues.

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EXAMPLE III

CD4 Chimeras Can Ass ciat With Other Rec ptor Chains

Cell surface expression of the macrophage/natural killer cell form of human $Fc\gamma RIII$ ($CD16_{TM}$) on

5 transfectants is facilitated by cotransfection with murine (Kurosaki et al., Nature, 342:805-807 (1989)) or human (Hibbs et al., Science, 246:1608-1611 (1989)) γ, as well as by human ζ (Lanier et al., Nature, 342:803-805 (1989)).

10 Consistent with these reports, expression of the chimeras also allowed surface expression of $CD16_{TM}$) when delivered to the target cell either by cotransfection or by coinfection with recombinant vaccinia viruses (Fig. 2). The promotion of ($CD16_{TM}$) surface expression by ζ was

15 more pronounced than promotion by γ (Fig. 2) in the cell lines examined, whereas native CD4 (data not shown) did not enhance $CD16_{TM}$ surface expression.

EXAMPLE IV

Asp ζ Mutants Do Not Coassociate with Fc Receptor

20 To create chimeras which would not associate with existing antigen or Fc receptors, mutant ζ fusion proteins which lacked either the intramembranous Asp or intramembranous Cys residue or both were prepared. Flow cytometry showed that the intensity of cell surface

25 expression by the different mutant chimeras was not appreciably different from the unmutated precursor (data not shown) and immunoprecipitation experiments showed that total expression by the chimeras was similar (Fig. 3). As expected, the mutant chimeras lacking the

30 transmembrane cysteine residue were found not to form disulfide linked dimers (Fig. 3). The two mutant chimeras lacking Asp were incapable of supporting the surface expression of $CD16^{TM}$, whereas the monomeric chimeras lacking Cys but bearing Asp allowed $CD16_{TM}$ to be

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coexpressed, but at lower efficiency than the parental
dimer (Fig. 3).

EXAMPLE V

Mutant Receptors Retain the Ability to Initiate a Calcium
5 **Response**

        To determine whether crosslinking of the fusion
proteins would allow the accumulation of free
intracellular calcium in a manner similar to that known
to occur with the T cell antigen receptor, cells of the
10 human T cell leukemia line, Jurkat E6 (ATCC Accessior
Number TIB 152, American Type Culture Collection,
Rockville, MD), were infected with the vaccinia
recombinants and the relative cytoplasmic calcium
concentration following crosslinking of the extracellular
15 domain with antibodies was measured. Flow cytometric
measurements were performed with cells loaded with the
calcium sensitive dye Indo-1 (Grynkiewicz et al., $\underline{J.}$
$\underline{Biol. Chem.}$, $\underline{260}$:3340-3450 (1985); Rabinovitch et al., $\underline{J.}$
$\underline{Immunol.}$, $\underline{137}$:952-961 (1986)). Figure 4 shows the
20 results of calcium flux experiments with cells infected
with CD4:ζ and the Asp⁻ and Cys⁻ mutants of ζ.
Crosslinking of the chimeras, reproducibly increased
intracellular calcium. CD4:η and CD4:γ similarly allowed
accumulation intracellular calcium in infected cells
25 (data not shown). Jurkat cells express low levels of CD4
on the cell surface, however, crosslinking of the native
CD4 in the presence or absence of CD16:ζ (C.R. and B.S.
unpublished) (Fig. 4 and data not shown) does not alter
intracellular calcium levels.

EXAMPLE VI

30

CD4:ζ, η, and γ Chimeras Mediate Cytolysis of Targets
Expressing HIV gp120/41
        To determine whether the chimeric receptors would
trigger cytolytic effector programs, a model

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target: ffector system based on CD4 recognition of the
HIV env lop gp120/gp41 complex was created. HeLa cells
were infected with recombinant vaccinia viruses
expressing gp120/gp41 (Chakrabarti et al., Nature,
5 320:535-537 (1986); Earl et al., J. Virol., 64:2448-2451
(1990)) and labeled with [51]Cr. The labeled cells were
incubated with cells from a human allospecific (CD8^{+}, CD4^{-}
) cytotoxic T lymphocyte line which had been infected
with vaccinia recombinants expressing the CD4:ζ, CD4:η,
10 or CD4:γ chimeras, or the CD4:ζCys11Gly:Asp15Gly double
mutant chimera. Fig. 5 shows that HeLa cells expressing
gp120/41 were specifically lysed by cytotoxic T
lymphocytes (CTL) expressing CD4 chimeras. Uninfected
HeLa cells were not targeted by CTL armed with CD4:ζ
15 chimeras, and HeLa cells expressing gp120/41 were not
recognized by uninfected CTL. To compare the efficacy of
the various chimeras, the effector to target ratios were
corrected for the fraction of CTL expressing CD4
chimeras, and for the fraction of HeLa cells expressing
20 gp120/41, as measured by flow cytometry. Fig. 5C shows a
cytometric analysis of CD4 expression by the CTL used in
the cytolysis experiment shown in Figs. 4A and 4B.
Although the mean density of surface CD4:ζ greatly
exceeded the mean density of CD4:η, the cytolytic
25 efficiencies of cells expressing either form were
similar. Correcting for the fraction of targets
expressing gp120, the efficiency of cytolysis mediated by
CD4:ζ and CD4:η proteins are comparable to the best
efficiencies reported for specific T cell receptor
30 target:effector pairs (the mean effector to target ratio
for 50% release by T cells expressing CD4:ζ was 1.9 ±
0.99, n=10). The CD4:γ fusion was less active, as was
the CD4:ζ fusion lacking the transmembrane Asp and Cys
residues. However in both cases significant cytolysis
35 was observed (Fig. 5).

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To control for the possibility that vaccinia
infection might promote artefactual recognition by CTL,
similar cytolysis experiments were performed with target
cells infected with vaccinia recombinants expressing the
5 phosphatidylinositol linked form of CD16 ($CD16_{PI}$) and
labeled with ^{51}Cr, and with CTL infected with control
recombinants expressing either $CD16_{PI}$ or CD16:ζ. Fig. 6A
shows that T cells expressing non-CD4 chimeras do not
recognize native HeLa cells or HeLa cells expressing
10 gp120/41, and similarly that T cells expressing CD4
chimeras do not recognize HeLa cells expressing other
vaccinia-encoded surface proteins. In addition, CTLs
expressing non-chimeric CD4 do not significantly lyse
HeLa cells expressing gp120/41 (Fig. 6A).

15   EXAMPLE VII
MHC Class II-Bearing Cells Are Not Targeted by the
Chimeras
        CD4 is thought to interact with a nonpolymorphic
sequence expressed by MHC class II antigen (Gay et al.,
20 Nature, 328:626-629 (1987); Sleckman et al., Nature,
328:351-353 (1987)). Although a specific interaction
between CD4 and class II antigen has never been
documented with purified proteins, under certain
conditions adhesion between cells expressing CD4 and
25 cells expressing class II molecules can be demonstrated
(Doyle et al., Nature, 330:256-259 (1987); Clayton et
al., J. Exp. Med., 172:1243-1253 (1990); Lamarre et al.,
Science, 245:743-746 (1989)). Next examined was whether
killing could be detected against cells bearing class II.
30 Fig. 6B shows that there is no specific cytolysis
directed by CD4:ζ against the Raji B cell line, which
expresses abundant class II antigen. Although a modest
(≈5%) cytolysis is observed, a class II-negative mutant
of Raji, RJ2.2.5, (Accolla, R.S., J. Exp. Med., 157:1053-

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1058 (1983)) shows a similar susceptibility, as do Raji
cells incubated with uninfected T cells.

EXAMPLE VIII
Sequence Requirements for Induction of Cytolysis by the T
5 Cell Antigen/Fc Receptor Zeta Chain

Although chimeras between CD4 and ζ can arm
cytotoxic T lymphocytes (CTL) to kill target cells
expressing HIV gp120, an alternative to CD4 was sought in
order to unambiguously compare the properties of zeta
10 chimeras introduced into human T cell lines. Such lines
can express CD4, making it difficult to specifically
define the relationship between the type or degree of
calcium mobilization and the cytotoxic potential of the
different chimeras. To circumvent this, chimeras were
15 created between ζ and CD16 in which the extracellular
domain of CD16 is attached to the transmembrane and
intracellular sequences of ζ (Fig. 7A). The gene fusions
were introduced into a vaccinia virus expression plasmid
bearing the E. coli gpt gene as a selectable marker and
20 inserted into the genome of the vaccinia WR strain by
homologous recombination and selection for growth in
mycophenolic acid (Falkner and Moss, J. Virol. 62:1849
(1988); Boyle and Coupar, Gene 65:123 (1988)).

T cell lines were infected with the vaccinia
25 recombinants and the relative cytoplasmic free calcium
ion concentration was measured following crosslinking of
the extracellular domains with antibodies. Both
spectrofluorimetric (bulk population) and flow cytometric
(single cell) measurements were performed, with cells
30 loaded with the dye Indo-1 (Grynkiewicz et al., J. Biol.
Chem. 260:3440 (1985); Rabinovitch et al., J. Immunol.
137:952 (1986)). Figure 7B shows an analysis of data
collected from cells of the Jurkat human T cell leukemia
line infected with vaccinia recombinants expressing

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CD16:ʃ fusion protein. Crosslinking of the chimeras
reproducibly incr ased intracellular calcium, while
similar treatment of cells expressing nonchimeric CD16
had little or no effect. When the chimera was expressed

5   in mutant cell lines lacking antigen receptor, either
REX33A (Breitmeyer et al. _J. Immunol._ **138**:726 (1987);
Sancho et al. _J. Biol. Chem_ **264**:20760 (1989)), or Jurkat
mutant JRT3.T3.5 (Weiss et al., _J. Immunol._ **135**:123
(1984)); or a strong response to CD16 antibody

10   crosslinking was seen. Similar data have been collected
on the REX20A (Breitmeyer et al., _supra_, 1987; Blumberg
et al., _J. Biol. Chem._ **265**:14036 (1990)) mutant cell
line, and a CD3/Ti negative mutant of the Jurkat cell
line established in this laboratory (data not shown).

15   Infection with recombinants expressing CD16:ʃ did not
restore the response to anti-CD3 antibody, showing that
the fusion protein did not act by rescuing intracellular
CD3 complex chains (data not shown).

     To evaluate the ability of the chimeras to
20   redirect cell-mediated immunity, CTLs were infected with
vaccinia recombinants expressing CD16 chimeras and used
to specifically lyse hybridoma cells expressing membrane-
bound anti-CD16 antibodies (see below). This assay is an
extension of a hybridoma cytotoxicity assay originally

25   developed to analyze effector mechanisms of cells bearing
Fc receptors (Graziano and Fanger, _J. Immunol._ **138**:945,
1987; Graziano and Fanger, _J. Immunol._ **139**:35-36, 1987;
Shen et al., _Mol. Immunol._ **26**:959, 1989; Fanger et al.,
Immunol. Today **10**: 92, 1989). Fig. 8B shows that

30   expression of CD16:ʃ in cytotoxic T lymphocytes allows
the armed CTL to kill 3G8 (anti-CD16; Fleit et al., _Proc._
Natl. Acad. Sci. USA **79**:3275, 1982) hybridoma cells,
whereas CTL expressing the phosphatidylinositol-linked
form of CD16 are inactive. CTL armed with CD16:ʃ also do

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not kill hybridoma cells expressing an irrelevant
antibody (data not shown).

     To identify the minimal ʃ sequences necessary for
cytolysis, a series of deletion mutants were prepared in
5 which successively more of the ʃ intracellular domain was
removed from the carboxyl terminus (Fig. 8A). Most of
the intracellular domain of zeta could be removed with
little consequence for cytolytic potential; the full
length chimera CD16:ʃ was essentially equal in efficacy
10 to the chimera deleted to residue 65, CD16:ʃAsp66* (Fig.
8B). A substantial decrease in cytotoxicity was observed
on deletion to ʃ residue 59 (chimera CD16:ʃGlu60*), and
further deletion to residue 50 resulted in slightly less
activity. However complete loss of activity was not
15 observed even when the intracellular domain was reduced
to a three residue transmembrane anchor (Fig. 8B).

     Because ʃ is a disulfide linked dimer, one
explanation for the retention of cytolytic activity was
that endogenous ʃ was forming heterodimers with the
20 chimeric ʃ deletion, thereby reconstituting activity. To
test this idea, ʃ residues 11 and 15 were changed from
Asp and Cys respectively to Gly (Cys11Gly/Asp15Gly), and
immunoprecipitations were carried out as follows.
Approximately 2 x 10^6 CV1 cells were infected for one
25 hour in serum free DME medium with recombinant vaccinia
at a multiplicity of infection (moi) of at least ten.
Six to eight hours post-infection, the cells were
detached from the plates with PBS/1mM EDTA and surface
labeled with 0.2 mCi ^{125}I per 2 x 10^6 cells using
30 lactoperoxidase and H_2O_2 by the method of Clark and
Einfeld (*Leukocyte Typing II*, pp. 155-167,
Springer=Verlag, NY, 1986). The labeled cells were
collected by centrifugation and lysed in 1% NP-40, 0.1%
SDS, 0.15M NaCl, 0.05M Tris, pH 8.0, 5mM $MgCl_2$, 5mM KCl,
35 0.2M iodoacetamide and 1mM PMSF. Nuclei were removed by

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centrifugation, and CD16 proteins were immunoprecipitated
with antibody 3G8 (Fl it et al., supra, 1982; Medarex)
and anti-mouse IgG agarose (Cappel, Durham, NC). Samples
were electrophoresed through an 8% polyacrylamide/SDS gel
5   under non-reducing conditions or through a 10% gel under
reducing conditions. These immunoprecipitations
confirmed that the CD16:ʃCys11Gly/Asp15Gly chimera did
not associate in disulfide-linked dimer structures.

The cytolytic activity of the mutant receptors was
10 also tested. The mutated chimera deleted to residue 65
(CD16:ʃCys11Gly/Asp15Gly/Asp66*) was, depending on the
conditions of assay, two to eight fold less active in the
cytolysis assay than the comparable unmutated chimera
(CD16:ʃAsp66*), which was usually within a factor of two
15 of, or indistinguishable in activity from, CD16:ʃ (Fig.
9B). The reduction in activity of the mutant chimeras is
comparable to the reduction seen with CD4 chimeras of
similar structure (see above) and is most likely
attributable to the lower efficiency of ʃ monomers
20 compared to dimers. In contrast, the Asp⁻, Cys⁻ mutated
chimera deleted to residue 59 had no cytolytic activity
(Fig. 9B), supporting the hypothesis that association
with other chains mediated by the transmembrane Cys
and/or Asp residues was responsible for the weak
25 persistence of cytolytic activity in deletions more amino
terminal than residue 65.

Flow cytometric studies showed that the deletion
mutants lacking transmembrane Asp and Cys residues could
still promote an increase in free intracellular calcium
30 ion in response to antibody crosslinking in a TCR⁻ mutant
Jurkat cell line (Fig. 9D). Similar results were
obtained for chimeras expressed in the parental Jurkat
line (not shown). In the case of
CD16:ʃCys11Gly/Asp15Gly/Glu60*, these data demonstrate
35 that the ability to mediate calcium responsiveness can be

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mutationally separated from the ability to support
cytolysis.

To definitively eliminate the possible
contribution of ζ transmembrane residues, the
5 transmembrane and first 17 cytoplasmic residues of ζ were
replaced by sequences encoding the membrane spanning and
first 14 or first 17 cytoplasmic residues of the CD5 or
CD7 antigens, respectively (Fig. 9A). The resulting
tripartite fusion proteins CD16:5:ζ(48-65) and
10 CD16:7:ζ(48-65) did not form disulfide-linked dimers as
do the simpler CD16:ζ chimeras, because they lacked the
cysteine residue in the ζ transmembrane domain. Both
tripartite chimeras were able to mobilize calcium in
Jurkat and TCR negative cell lines (Fig. 9D) and to mount
15 a cytolytic response in CTL (Fig. 9C and data not shown).
However truncation of the ζ portion to residue 59 in
chimera CD16:7:ζ(48-59) abrogates the ability of
tripartite fusion to direct calcium responsiveness in TCR
positive or negative Jurkat cells or cytolysis in mature
20 CTL (Fig. 9C and 9D and data not shown).

To examine the contributions of individual
residues within the 18-residue motif, we prepared a
number of mutant variants by site-directed mutagenesis,
and evaluated their ability to mediate receptor-directed
25 killing under conditions of low (Figs. 10A and 10D) or
high (Figs. 10B and 10E) expression of chimeric receptor.
Fig. 10 shows that while a number of relatively
conservative substitutions (i.e., replacing acidic
residues with their cognate amides, or tyrosine with
30 phenylalanine) which spanned residues 59 to 63 yielded
moderate compromise of cytolytic efficacy, in general the
variants retained the ability to mobilize calcium.
However collectively these residues comprise an important
submotif inasmuch as their deletion eliminates cytolytic
35 activity. Conversion of Tyr 62 to either Phe or Ser

eliminated both the cytotoxic and calcium responses. At
the amino terminus of the 18 residue segment, replacement
of Tyr 51 with Phe abolished both calcium mobilization
and cytolytic activity, while substitution of Leu with
5 Ser at position 50 eliminated the calcium response while
only partially impairing cytolysis. Without being bound
to a particular hypothesis, it is suspected that the
inability of the Leu50Ser mutant to mobilize calcium in
short term flow cytometric assays does not fully reflect
10 its ability to mediate a substantial increase in free
intracellular calcium ion over the longer time span of
the cytolysis assay. However, calcium-insensitive
cytolytic activity has been reported for some cytolytic T
cell lines, and the possibility that a similar phenomenon
15 underlies the results described herein has not been ruled
out. Replacement of Asn48 with Ser partially impaired
cytotoxicity in some experiments while having little
effect in others.

To investigate the potential role of redundant
20 sequence elements, the intracellular domain of ς was
divided into three segments, spanning residues 33 to 65,
71 to 104, and 104 to 138. Each of these segments was
attached to a CD16:CD7 chimera by means of a MluI site
introduced just distal to the basic membrane anchoring
25 sequences of the intracellular domain of CD7 (see below;
Fig. 11A). Comparison of the cytolytic efficacy of the
three elements showed they were essentially equipotent
(Fig. 11B). Sequence comparison (Fig. 11A) shows that
the second motif bears eleven residues between tyrosines,
30 whereas the first and third motifs bear ten.

Although a precise accounting of the process of T
cell activation has not been made, it is clear that
aggregation of the antigen receptor, or of receptor
chimeras which bear ς intracellular sequences, triggers
35 calcium mobilization, cytokine and granule release, and

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the appearance of cell surface markers of activation.
The active site of ς, a short linear peptide sequence
probably too small to have inherent enzymatic activity,
likely interacts with one or at most a few proteins to
5 mediate cellular activation. It is also clear that
mobilization of free calcium is not by itself sufficient
for cellular activation, as the ability to mediate
cytolysis can be mutationally separated from the ability
to mediate calcium accumulation.

10   As shown herein, addition of 18 residues from the
intracellular domain of ς to the transmembrane and
intracellular domain of two unrelated proteins allows the
resulting chimeras to redirect cytolytic activity against
target cells which bind to the extracellular portion of
15 the fusion proteins. Although chimeras bearing the 18
residue motif are approximately eight-fold less active
than chimeras based on full length ς, the reduced
activity can be attributed to the loss of transmembrane
interactions which normally allow wild-type ς to form
20 disulfide linked dimers. That is, ς deletion constructs
which have the same carboxyl terminus as the motif and
lack transmembrane Cys and Asp residues typically show
slightly less activity than chimeras bearing only the 18
residue motif.

25   The cytolytic competency element on which we have
focused has two tyrosines and no serines or threonines,
restricting the possible contributions of phosphorylation
to activity. Mutation of either tyrosine destroys
activity, however, and although preliminary experiments
30 do not point to a substantial tyrosine phosphorylation
following crosslinking of chimeric surface antigens
bearing the 18 reside motif, the possible participation
of such phosphorylation at a low level cannot be
excluded. In addition to the effects noted at the two
35 tyrosine residues, a number of amino acid replacements at

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the amino and carboxyl terminus of the motif weaken
activity under conditions of low receptor density.

    Sequences similar to the ζ active motif can be
found in the cytoplasmic domains of several other
5 transmembrane proteins, including the CD3 δ and γ
   molecules, the surface IgM associated proteins mb1 and
   B29, and the β and γ chains of the high affinity IgE
   receptor, FcεRI (Reth, Nature 338:383, 1989). Although
   the function of these sequences is uncertain, if
10 efficiently expressed, each may be capable of autonomous
   T cell activation, and such activity may explain the
   residual TCR responsiveness seen in a zeta-negative
   mutant cell line (Sussman et al., Cell 52:85, 1988).

    ζ itself bears three such sequences, approximately
15 equally spaced, and a rough trisection of the
   intracellular domain shows that each is capable of
   initiating a cytolytic response. η, a splice isoform of
   ζ (Jin et al., supra, 1990; Clayton et al., Proc. Natl.
   Acad. Sci. USA 88:5202, 1991), lacks the carboxyl half of
20 the third motif. Because removal of the carboxyl half of
   the first motif abolishes activity, it appears likely
   that the majority of the biological effectiveness of η
   can be attributed to the first two motifs. Although by
   different measures η is equally as active as ζ in
25 promoting antigen-mediated cytokine release (Bauer et
   al., Proc. Natl. Acad. Sci. USA 88:3842, 1991) or
   redirected cytolysis (see above), η is not phosphorylated
   in response to receptor stimulation (Bauer et al., supra,
   1991). Thus either the presence of all three motifs is
30 required for phosphorylation, or the third motif
   represents a favored substrate for an unidentified
   tyrosine kinase.

EXAMPLE IX

Cytolytic Signal Transduction by Human Fc Receptor

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To evaluate the actions of different human Fc
receptor subtyp s, chimeric molecules were created in
which the extracellular domain of the human CD4, CD5 or
CD16 antigens were joined to the transmembrane and
5 intracellular domains of the FcRIIγA, B1, B2, and C
subtypes (nomenclature of Ravetch and Kinet, Ann. Rev.
Immunol. 9:457, 1991). Specifically, cDNA sequences
corresponding to the transmembrane and cytoplasmic
domains of the previously described FcRIIA, B1, and B2
10 isoforms were amplified from the preexisting clone PC23
or from a human tonsil cDNA library (constructed by
standard techniques) using oligonucleotide primers:
        CCC GGA TCC CAG CAT GGG CAG CTC TT (SEQ ID NO: 18;
FcRIIA forward)
15   CGC GGG GCG GCC GCT TTA GTT ATT ACT GTT GAC ATG
GTC GTT (SEQ ID NO: 19; FcRIIA reverse);
        GCG GGG GGA TCC CAC TGT CCA AGC TCC CAG CTC TTC
ACC G (SEQ ID NO: 20; FcRIIB1 and FcRIIB2 forward); and
        GCG GGG GCG GCC GCC TAA ATA CGG TTC TGG TC (SEQ ID
20 NO: 21; FcRIIB1 and FcRIIB2 reverse).
These primers contained cleavage sites for the enzymes
BamHI and NotI, respectively, indented 6 residues from
the 5' end. The NotI site was immediately followed by an
antisense stop codon, either CTA or TTA. All primers
25 contained 18 or more residues complementary to the 5' and
3' ends of the desired fragments. The cDNA fragment
corresponding to the FcRIIγC cytoplasmic domain, which
differs from the IIA isoform in only one amino acid
residue (L for P at residue 268) was generated by site
30 directed mutagenesis by overlap PCR using primers of
sequence:
        TCA GAA AGA GAC AAC CTG AAG AAA CCA ACA A (SEQ ID
NO: 22) and
        TTG TTG GTT TCT TCA GGT TGT GTC TTT CTG A (SEQ ID
35 NO: 23).

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The PCR fragments were inserted into vaccinia virus
expression vectors which contained the CD16 or CD4
extracellular domains respectively and subsequently
inserted into wild type vaccinia by recombination at the
5 thymidine kinase locus, using selection for cointegration
of E coli gpt to facilitate identification of the desired
recombinants. The identities of all isoforms (shown in
Fig. 12) were confirmed by dideoxy sequencing.

    Production of the chimeric receptor proteins was
10 further confirmed by immunoprecipitation studies.
Approximately 10^7 JRT3.T3.5 cells were infected for one
hour in serum free IMDM medium with recombinant vaccinia
at a multiplicity of infection of at least ten. Twelve
hours post-infection, the cells were harvested and
15 surface labeled with 0.5mCi ^{125}I per 10^7 cells using the
lactoperoxidase/glucose oxidase method (Clark and
Einfeld, supra). The labeled cells were collected by
centrifugation and lysed 1% NP-40, 0.1mM $MgCl_2$, 5mM KCl,
0.2M iodoacetamide and 1mM PMSF. Nuclei were removed by
20 centrifugation, and CD16 fusion proteins
immunoprecipitated with antibody 4G8 and anti-mouse IgG
agarose. Samples were electrophoresed under reducing
conditions. All immunoprecipitated chimeric receptor
molecules were of the expected molecular masses.

25   To test the ability of the chimeric receptors to
mediate an increase in cytoplasmic free calcium ion, the
recombinant viruses were used to infect the TCR⁻ mutant
Jurkat cell line JRT3.T3.5 (as described herein) and
cytoplasmic free calcium was measured in the cells (as
30 described herein) following crosslinking of the receptor
extracellular domains with monoclonal antibody 3G8 or
Leu-3A (as described herein). These experiments revealed
that the intracellular domains of FcRγII A and C were
capable of mediating an increase in cytoplasmic free
35 calcium ion after crosslinking of the extracellular

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domains, whereas the intracellular domains of FcRγII B1
and B2 w re inactive under comparable conditi ns (Fig. 13
A and 13B). The CD4, CD5 and CD16 hybrids of FcRγII A
shared essentially equal capacity to promote the calcium
5   response (Fig. 13 and data not shown). Other cell lines,
from both monocytic and lymphocytic lineages, were
capable of responding to the signal initiated by
crosslinking of the extracellular domains (data not
shown).

10   To explore the involvement of the different FcRγII
intracellular domains in cytolysis, human cytotoxic T
lymphocytes (CTL) were infected with vaccinia
recombinants expressing CD16:FcRγII A, B1, B2 and C
chimeras. The infected cells were then cocultured with
15 ^{51}Cr-loaded hybridoma cells (i.e., 3G8 10-2 cells) which
expressed cell surface antibody to CD16. In this assay
CTLs bearing the CD16 chimera killed the hybridoma target
cells (allowing release of free ^{51}Cr) if the CD16
extracellular domain of the chimera has been joined to an
20 intracellular segment capable of activating the
lymphocyte effector program; this cytolysis assay is
described in detail below. Fig. 14A shows that CTL armed
with CD16:FcRγIIA and C, but not FcRγII B1 or B2, are
capable of lysing target cells expressing cell surface
25 anti-CD16 antibody.

To eliminate the possibility that the specific
cytolysis was in some way attributable to interaction
with the CD16 moiety, cytolysis experiments were
conducted in which the FcRII intracellular domains were
30 attached to a CD4 extracellular domain. In this case the
target cells were HeLa cells expressing HIV envelope
gp120/41 proteins (specifically, HeLa cells infected with
the vaccinia vector vPE16 (available from the National
Institute of Allergy and Infections Disease AIDS
35 Depository, Bethesda, MD). As in the CD16 system, target

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cells expressing HIV envelope were susceptible to lysis
by T cells xpr ssing the CD4:FcRγII A chimera, but n t
FcRγII B1 or B2 (Fig. 14B).

    The intracellular domains of FcRγII A and C share
5 no appreciable sequence homology with any other protein,
including the members of the extended FcRγ/TCRζ family.
To define the sequence elements responsible for induction
of cytolysis, 5' and 3' deletions of the intracellular
domain coding sequences (described below and shown in
10 Fig. 15A) were prepared and were evaluated for efficacy
in calcium mobilization and cytolysis assays (as
described herein). In the experiments in which the amino
terminal portion of the intracellular domain was removed,
the transmembrane domain of FcRγII was replaced with the
15 transmembrane domain of the unrelated CD7 antigen to
eliminate the possible contribution of interactions
mediated by the membrane-spanning domain.

    Figs. 15B and 15C show that removal of the 14
carboxyl-terminal residues, including tyrosine 298,
20 resulted in a complete loss of cytolytic capacity and a
substantial reduction in calcium mobilization potential.
Further deletion to just before tyrosine 282 gave an
identical phenotype (Figs. 15B and 15C). Deletion from
the N-terminus of the intracellular domain to residue 268
25 had no substantial effect on either calcium profile or
cytolytic potency, whereas deletion to residue 275
markedly impaired free calcium release but had little
effect on cytolysis (Figs. 15D and 15E). Further
deletion, to residue 282, gave FcRγII tails which lacked
30 the ability to either mobilize calcium or trigger
cytolysis (Figs. 15D and 15E). The 'active element'
defined by these crude measures is relatively large (36
amino acids) and contains two tyrosines separated by 16
residues.

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EXAMPLE X

Other intracellular and transmembrane signal transducing domains according to the invention may be derived from the T cell receptor proteins, CD3 delta and
5 T3 gamma, and the B cell receptor proteins, mb1 and B29. The amino acid sequences of these proteins is shown in Fig. 16 (CD3 delta; SEQ ID NO: 24), Fig. 17 (T3 gamma; SEQ ID NO: 25), Fig. 18 (mb1; SEQ ID NO: 26) and Fig. 19 (B29; SEQ ID NO: 27). The portions of the sequences
10 sufficient for cytolytic signal transduction (and therefore preferably included in a chimeric receptor of the invention) are shown in brackets. Chimeric receptors which include these protein domains are constructed and used in the therapeutic methods of the invention
15 generally as described above.

EXAMPLE XI

Experimental Methods

Vaccinia Infection and Radioimmunoprecipitation

Approximately 5×10^6 CV1 cells were infected for
20 one hour in serum free DME medium with recombinant vaccinia at a multiplicity of infection (moi) of at least ten (titer measured on CV1 cells). The cells were placed in fresh medium after infection and labelled metabolically with 200μCi/ml ^{35}S-methionine plus cysteine
25 (Tran^{35}S-label, ICN; Costo Mesa, CA) in methionine and cysteine free DMEM (Gibco; Grand Island, NY) for six hours. The labelled cells were detached with PBS containing 1mM EDTA, collected by centrifugation, and lysed in 1% NP-40, 0.1% SDS, 0.15 M NaCl, 0.05M Tris pH
30 8.0, 5mM EDTA, and 1mM PMSF. Nuclei were removed by centrifugation, and CD4 proteins immunoprecipitated with OKT4 antibody and anti-mouse IgG agarose (Cappel, Durham, NC). Samples were electrophoresed through 8% polyacrylamide/SDS gels under non-reducing (NR) and
35 reducing (R) conditions. Gels containing ^{35}S-labelled

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samples were impregnated with En^{3}Hance (New England
Nucl ar, Boston, MA) prior to autoradiography.
Facilitated expression of the transmembrane form of CD16,
CD16$_{TM}$, was measured by comparing its expression in CV1

5 cells singly infected with CD16$_{TM}$ with expression in cells
coinfected with viruses encoding CD16$_{TM}$ and ς or γ
chimeras. After infection and incubation for six hours
or more, cells were detached from plates with PBS, 1mM
EDTA and the expression of CD16TM or the chimeras was

10 measured by indirect immunofluorescence and flow
cytometry.

Calcium Flux Assay

        Jurkat subline E6 (Weiss et al., _J. Immunol._,
_133_:123-128 (1984)) cells were infected with recombinant

15 vaccinia viruses for one hour in serum free IMDM at an
moi of 10 and incubated for three to nine hours in IMDM,
10% FBS. Cells were collected by centrifugation and
resuspended at 3 x 10^6 cells/ml in complete medium
containing 1mM Indo-1 acetomethoxyester (Grynkiewicz et

20 al., _J. Biol. Chem._, _260_:3340-3450 (1985)) (Molecular
Probes) and incubated at 37°C for 45 minutes. The Indo-
1 loaded cells were pelleted and resuspended at 1 x
10^6/ml in serum free IMDM and stored at room temperature
in the dark. Cells were analyzed for free calcium ion by

25 simultaneous measurement of the violet and blue
fluorescence emission by flow cytometry (Rabinovitch et
al., _J. Immunol._, _137_:952-961 (1986)). To initiate
calcium flux, either phycoerythrin (PE)-conjugated Leu-3A
(anti-CD4) (Becton Dickinson, Lincoln Park, NJ) at 1

30 μg/ml was added to the cell suspension followed by
10μg/ml of unconjugated goat anti-mouse IgG at time 0 or
unconjugated 3G8 (anti-CD16) monoclonal antibody was
added to the cell suspension at 1 μg/ml followed by 10
μg/ml of PE-conjugated Fab$_2$' goat anti-monse IgG at time

35 0. Histograms of the violet/blue emission ratio were

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collected from the PE positive (infected) cell
population, which typically r presented 40-80% of all
cells. The T cell antigen receptor response in
uninfected cells was triggered by antibody OKT3, without
5 crosslinking. For experiments involving CD16 chimeric
receptors, samples showing baseline drift toward lower
intracellular calcium (without antibody) were excluded
from the analysis. Histogram data were subsequently
analyzed by conversion of the binary data to ASCII using
10 Write Hand Man (Cooper City, FL) software, followed by
analysis with a collection of FORTRAN programs. The
violet/blue emission ratio prior to the addition of the
second antibody reagents was used to establish the
normalized initial ratio, set equal to unity, and the
15 resting threshold ratio, set so that 10% of the resting
population would exceed threshold.

Cytolysis Assay

    Human T cell line WH3, a $CD8^+$ $CD4^-$ HLA B44
restricted cytolytic line was maintained in IMDM, 10%
20 human serum with 100 U/ml of IL-2 and was periodically
stimulated either nonspecifically with irradiated (3000
rad) HLA-unmatched peripheral blood lymphocytes and
1μg/ml of phytohemagglutinin, or specifically, with
irradiated B44-bearing mononuclear cells. After one day
25 of nonspecific stimulation, the PHA was diluted to 0.5
μg/ml by addition of fresh medium, and after three days
the medium was changed. Cells were grown for at least 10
days following stimulation before use in cytotoxicity
assays. The cells were infected with recombinant
30 vaccinia at a multiplicity of infection of at least 10
for one hour in serum free medium, followed by incubation
in complete medium for three hours. Cells were harvested
by centrifugation and resuspended at a density of 1 x 10^7
cells/ml. 100μl were added to each well of a U-bottom
35 microtiter plate containing 100 μl/well of complete

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medium. Cells were diluted in two-fold serial steps.
Two wells for each sample did not contain lymphocytes, to
allow spontaneous chromium release and total chromium
uptake to be measured. The target cells, from HeLa
5 subline S3, were infected in 6.0 or 10.0 cm plates at an
approximate moi of 10 for one hour in serum free medium,
followed by incubation in complete medium for three
hours. They were then detached from the dishes with PBS,
1mM EDTA and counted. An aliquot of 10^6 target cells
10 (HeLa, Raji, or RJ2.2.5 cells for the CD4 chimeric
receptor experiments and 3G8 10-2 cells; Shen et al.,
Mol. Immunol. 26:959 (1989) for the CD16 chimeric
receptor experiments) was centrifuged and resuspended in
50 μl of sterile ^{51}Cr-sodium chromate (1mCi/ml, Dupont
15 Wilmington, DE) for one hour at 37°C with intermittent
mixing, then washed three times with PBS. 100 μl of
labelled cells resuspended in medium at 10^5 cells/ml were
added to each well. Raji and RJ2.2.5 target cells were
labelled in the same manner as HeLa cells. The
20 microtiter plate was spun at 750 x g for 1 minute and
incubated for 4 hours at 37°C. At the end of the
incubation period, the cells in each well were
resuspended by gentle pipetting, a sample removed to
determine the total counts incorporated, and the
25 microtiter plate spun at 750 x g for 1 minute. 100μl
aliquots of supernatant were removed and counted in a
gamma ray scintillation counter. The percent killing was
corrected for the fraction of infected target cells
(usually 50-90%) measured by flow cytometry. For
30 infected effector cells the effector:target ratio was
corrected for the percent of cells infected (usually 20-
50% for the CD4 chimeric receptor experiments and >70%
for the CD16 chimeric receptor experiments).

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In Vitr Mutagenesis f th ʓ Sequ nc

To create point mutations in amino acid residues
11 and or 15 of th ʓ sequence, synthetic oligonucleotide
primers extending from the BamHI site upstream of the ʓ

5 transmembrane domain, and converting native ʓ residue 11
from Cys to Gly (C11G) or residue 15 from Asp to Gly
(D15G) or both (C11G/D15G) were prepared and used in PCR
reactions to generate mutated fragments which were
reinserted into the wild type CD4:ʓ constructs.

10 To create ʓ deletions, ʓ cDNA sequences were
amplified by PCR using synthetic oligonucleotide primers
designed to create a stop codon (UAG) after residues 50,
59, or 65. The primers contained the cleavage site for
the enzyme NotI indented five or six residues from the 5'

15 end, usually in a sequence of the form CGC GGG CGG CCG
CTA (SEQ ID NO: 11), where the last three residues
correspond to the stop anticodon. The NotI and stop
anticodon sequences were followed by 18 or more residues
complementary to the diesired 3' end of the fragment.

20 The resulting chimeras were designated CD16:ʓY51*,
CD16:ʓE60* and CD16:ʓD66* respectively. The BamHI site
upstream of the transmembrane domain and the NotI site
were used to generate fragments that were reintroduced
into the wild type CD16:ʓ construct. Monomeric ʓ

25 chimeras were created by liberating the ʓ transmembrane
and membrane proximal intracellular sequences by BamHI
and SacI digestion of the Asp⁻ and Cys⁻ CD4:ʓ construct
described above and inserting the fragment into the
CD16:ʓE60* and CD16:ʓD66* construct respectively.

30 **CD16:7:ʓ(48-65) and CD16:7ʓ(48-59) tripartite chimera
construction.**

To prepare the construct CD16:ʓD66*, the ʓ cDNA
sequence corresponding to the transmembrane domain and
the 17 following residues of the cytoplasmic domain was

35 replaced by corresponding transmembrane and cytoplasmic

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domain obtained from the CD5 and CD7 cDNA. The CD5 and
CD7 fragments were generated by a PCR reaction using
forward oligonucleotides including a BamHI restriction
cleavage site and corresponding to the region just
5   upstream of the transmembrane domain of CD5 and CD7
respectively and the following reverse oligonucleotides
overlapping the CD5 and CD7 sequences respectively and
the ς sequence which contained the SacI restriction
cleavage site.
10   CD5:ς: CGC GGG CTC GTT ATA GAG CTG GTT CTG GCG
CTG CTT CTT CTG (SEQ ID NO: 12)
           CD7:ς: CGC GGG GAG CTC GTT ATA GAG CTG GTT TGC
CGC CGA ATT CTT ATC CCG (SEQ ID NO: 13).
The CD5 and CD7 PCR products were digested with BamHI and
15   SacI and ligated to BamHI and SacI digested CD16:ςE60*
and replacing the ς sequence from BamHI to SacI by the
CD7 fragment. To make the constructs CD16:CD5 and
CD16:CD7, CD5 and CD7 fragments were obtained by PCR
using an oligonucleotide containing a NotI restriction
20   cleavage site and encoding a stop codon (UAA) after the
residue Gln416 and Ala193 of CD5 and CD7 respectively.
The CD5 and CD7 PCR fragment were digested with BamHI and
NotI and inserted in the CD16:ςAsp66* construct.
**In Vitro Mutagenesis of the N-terminal Residues within
25 the ς Cytolytic Signal-Transducing Motif**
           Synthetic oligonucleotide primers extending from
the SacI site inside the ς motif and converting native
residue 48 from Asn to Ser (N48S), residue 50 from Leu to
Ser (L50S) and residue 51 from Tyr to Phe (Y51F) were
30   synthesized and used in a PCR reaction to generate
fragments that were reintroduced into the wild type
CD16:7:ς(48-65) construct.
**In Vitro Mutagenesis of C-terminal Residues within the ς
Cytolytic Signal-Transducing Motif**

Synth tic oligonucleotide primers extending from
the NotI site 3' to the stop codon and converting native
residue 60 from Glu to Gln (E60Q), residue 61 from Glu to
Gln (E61Q), residue 62 from Tyr to Phe or Ser (Y62F or

5 Y62S) and residue 63 from Asp to Asn (D63N) were
synthesized and used in PCR to generate fragments that
were subcloned into the wild type CD16:ʃD66* construct
from the BamHI site to the NotI site.
CD16:7:ʃ(33-65), CD16:7:ʃ(71-104), CD16:7:ʃ(104-137)

10 **Chimera Constructions**

A CD7 transmembrane fragment bearing MluI and NotI
sites at the junction between the transmembrane and
intracellular domains was obtained by PCR using an
oligonnucleotide with the following sequence: CGC GGG

15 GCG GCC ACG CGT CCT CGC CAG CAC ACA (SEQ ID NO: 14). The
resulting PCR fragment was digested with BamHI and NotI
and reinserted into the CD16:7:ʃ(48-65) construct. ʃ
fragments encoding residues 33 to 65, 71 to 104, and 104
to 137 were obtained by PCR reaction using pairs of

20 primers containing MluI sites at the 5' end of the
forward primers and stop codons followed by NotI sites at
the 5' end of the reverse primers. In each case the
restriction sites were indented six residues from the 5'
terminus of the primer to insure restriction enzyme

25 cleavage.

ʃ 33: CGC GGG ACG CGT TTC AGC CGT CCT CGC CAG CAC
ACA (SEQ ID NO: 15);

ʃ 71: CGC GGG ACG CGT GAC CCT GAG ATG GGG GGA AAG
(SEQ ID NO: 16); and

30   ʃ 104: CGC GGG ACG CGT ATT GGG ATG AAA GGC GAG CGC
(SEQ ID NO: 17).

Construction of FcRγIIA Deletion Mutants

Carboxyl terminal FcRIIA deletion mutants were
constructed by PCR in the same fashion as for the full

35 length constructs, converting the sequences encoding

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tyrosine at positions 282 and 298 into stop codons (TAA).
The N-terminal deletions were generated by amplifying
fragments encoding successively less of the intracellular
domain by PCR, using oligonucleotides which allowed the
5   resulting fragments to be inserted between MluI and NotI
restriction sites into a previously constructed
expression plasmid encoding the CD16 extracellular domain
fused to the CD7 transmembrane domain, the latter
terminating in a MluI site ant the juncition between the
10 transmembrane and the intracellular domain.

OTHER EMBODIMENTS

The examples described above demonstrate that
aggregation of ς, η, or γ chimeras suffices to initiate
the cytolytic effector cell response in T cells. The
15 known range of expression of ς, η, and γ, which includes
T lymphocytes, natural killer cells, basophilic
granulocytes, macrophages and mast cells, suggests that
conserved sequence motifs may interact with a sensory
apparatus common to cells of hematopoietic origin and
20 that an important component of host defense in the immune
system may be mediated by receptor aggregation events.

The potency of the cytolytic response and the
absence of a response to target cells bearing MHC class
II receptors demonstrates that chimeras based on ς, η, or
25 γ form the basis for a genetic intervention for AIDS
through adoptive immunotherapy. The broad distribution
of endogenous ς and γ and evidence that Fc receptors
associated with γ mediate cytotoxicity in different cells
types (Fanger et al., Immunol. Today, 10:92-99 (1989))
30 allows a variety of cells to be considered for this
purpose. For example, neutrophilic granulocytes, which
have a very short lifespan (≈ 4h) in circulation and are
intensely cytolytic, are attractive target cells for
expression of the chimeras. Infection of neutrophils
35 with HIV is not likely to result in virus release, and

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the abundance of these cells (the most prevalent of the
leukocytes) should facilitate host defens . Anoth r
attractive possiblity for host cells are mature T cells,
a population presently accessible to retroviral

5   engineering (Rosenberg, S.A. _Sci. Am._, _262_:62-69 (1990)).
With the aid of recombinant IL-2, T cell populations can
be expanded in culture with relative ease, and the
expanded populations typically have a limited lifespan
when reinfused (Rosenberg et al., _N. Engl. J. Med._,

10 _323_:570-578 (1990)).

        Under the appropriate conditions, HIV recognition
by cells expressing CD4 chimeras should also provide
mitogenic stimuli, allowing the possibility that the
armed cell population could respond dynamically to the

15 viral burden. Although we have focused here on the
behavior of the fusion proteins in cytolytic T
lymphocytes, expression of the chimeras in helper
lymphocytes might provide an HIV-mobilized source of
cytokines which could counteract the collapse of the

20 helper cell subset in AIDS. Recent description of
several schemes for engineering resistance to infection
at steps other than virus penetration (Friedman et al.,
Nature, _335_:452-454 (1988); Green et al., _Cell_, _58_:215-
223 (1989); Malim et al., _Cell_, _58_:205-214 (1989); Trono

25 et al., _Cell_, _59_:113-120 (1989); Buonocore et al.,
Nature, _345_:625-628 (1990)) suggests that cells bearing
CD4 chimeras could be designed to thwart virus production
by expression of appropriate agents having an
intracellular site of action.

30   The ability to transmit signals to T lymphocytes
through autonomous chimeras also provides the ability for
the regulation of retrovirally engineered lymphocytes _in
vivo_. Crosslinking stimuli, mediated for example by
specific IgM antibodies engineered to remove complement-

35 binding domains, may allow such lymphocytes to increase

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in number in situ, while treatment with similar sp cific
IgG antibodies (f r example recognizing an amino acid
variation engineered into the chimeric chain) could
selectively deplete the engineered population.

5   Additionally, anti-CD4 IgM antibodies do not require
    additional crosslinking to mobilize calcium in Jurkat
    cells expressing CD4:ζ chimeras (data not shown). The
    ability to regulate cell populations without recourse to
    repeated extracorporeal amplification may substantially

10 extend the range and efficacy of current uses proposed
    for genetically engineered T cells.

        To create other chimeras consisting of ζ, η or γ
    intracellular sequences, cDNA or genomic sequences
    encoding an extracellular domain of the receptor can be

15 endowed with a restriction site introduced at a location
    just preceding the transmembrane domain of choice. The
    extracellular domain fragment terminating in the
    restriction site can then be joined to ζ, η, or γ
    sequences. Typical extracellular domains may be derived

20 from receptors which recognize complement, carbohydrates,
    viral proteins, bacteria, protozoan or metazoan
    parasites, or proteins induced by them. Similarly,
    ligands or receptors expressed by pathogens or tumor
    cells can be attached to ζ, η, or γ sequences, to direct

25 immune responses against cells bearing receptors
    recognizing those ligands.

        While the invention has been described in
    connection with specific embodiments thereof, it will be
    understood that it is capable of further modifications

30 and this application is intended to cover variations,
    uses, or adaptations of the invention and including such
    departures from the present disclosure as come within the
    art to which the invention pertains and as may be applied
    to the essential features hereinbefore set forth as

35 follows in the scope of the appended claims.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

    (i) APPLICANTS: The General Hospital Corporation

    (ii) TITLE OF INVENTION: Redirection of Cellular Immunity by Receptor
                   Chimeras

    (iii) NUMBER OF SEQUENCES: 27

    (iv) CORRESPONDENCE ADDRESS:
        (A) ADDRESSEE: Fish & Richardson
        (B) STREET: 225 Franklin Street
        (C) CITY: Boston
        (D) STATE: MA
        (E) COUNTRY: USA
        (F) ZIP: 02110-2804

    (v) COMPUTER READABLE FORM:
        (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb
        (B) COMPUTER: IBM PS/2 Model 50Z or 55SX
        (C) OPERATING SYSTEM: IBM P.C. DOS (Version 3.30)
        (D) SOFTWARE: Wordperfect (Version 5.0)

    (vi) CURRENT APPLICATION DATA:
        (A) APPLICATION NUMBER:
        (B) FILING DATE:
        (C) CLASSIFICATION:

    (vii) PRIOR APPLICATION DATA:
        (A) APPLICATION NUMBER: 07/665,961
        (B) FILING DATE: March 7, 1991
        (C) CLASSIFICATION:

    (viii) ATTORNEY/AGENT INFORMATION:
        (A) NAME: Clark, Paul T.
        (B) REGISTRATION NUMBER: 30,162
        (C) REFERENCE/DOCKET NUMBER: 00786/119002

    (ix) TELECOMMUNICATION INFORMATION:
        (A) TELEPHONE: (617) 542-5070
        (B) TELEFAX: (617) 542-8906
         (C) TELEX: 200154

(2) INFORMATION FOR SEQ ID NO:1:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1728 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

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(ii) MOLECULE TYPE: DNA (genomic)

(xi) SEQUENCE DESCRIPTI N: SEQ ID NO:1:

```
ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC         50

GCTCCTCCCA GCAGCCACTC AGGGAAACAA AGTGGTGCTG GGCAAAAAAG        100

GGGATACAGT GGAACTGACC TGTACAGCTT CCCAGAAGAA GAGCATACAA        150

TTCCACTGGA AAAACTCCAA CCAGATAAAG ATTCTGGGAA ATCAGGGCTC        200

CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGCT GACTCAAGAA        250

GAAGCCTTTG GGACCAAGGA AACTTCCCCC TGATCATCAA GAATCTTAAG        300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGAGGACC AGAAGGAGGA        350

GGTGCAATTG CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC        400

TTCAGGGGCA GAGCCTGACC CTGACCTTGG AGAGCCCCCC TGGTAGTAGC        450

CCCTCAGTGC AATGTAGGAG TCCAAGGGGT AAAAACATAC AGGGGGGGAA        500

GACCCTCTCC GTGTCTCAGC TGGAGCTCCA GGATAGTGGC ACCTGGACAT        550

GCACTGTCTT GCAGAACCAG AAGAAGGTGG AGTTCAAAAT AGACATCGTG        600

GTGCTAGCTT TCCAGAAGGC CTCCAGCATA GTCTATAAGA AGAGGGGGA        650

ACAGGTGGAG TTCTCCTTCC CACTCGCCTT TACAGTTGAA AAGCTGACGG        700

GCAGTGGCGA GCTGTGGTGG CAGGCGGAGA GGGCTTCCTC CTCCAAGTCT        750

TGGATCACCT TTGACCTGAA GAACAAGGAA GTGTCTGTAA AACGGGTTAC        800

CCAGGACCCT AAGCTCCAGA TGGGCAAGAA GCTCCCGCTC CACCTCACCC        850

TGCCCCAGGC CTTGCCTCAG TATGCTGGCT CTGGAAACCT CACCCTGGCC        900

CTTGAAGCGA AAACAGGAAA GTTGCATCAG GAAGTGAACC TGGTGGTGAT        950

GAGAGCCACT CAGCTCCAGA AAAATTTGAC CTGTGAGGTG TGGGGACCCA       1000

CCTCCCCTAA GCTGATGCTG AGCTTGAAAC TGGAGAACAA GGAGGCAAAG       1050

GTCTCGAAGC GGGAGAAGCC GGTGTGGGTG CTGAACCCTG AGGCGGGGAT       1100

GTGGCAGTGT CTGCTGAGTG ACTCGGGACA GGTCCTGCTG GAATCCAACA       1150

TCAAGGTTCT GCCCACATGG TCCACCCCGG TGCACGCGGA TCCCAAACTC       1200

TGCTACTTGC TAGATGGAAT CCTCTTCATC TACGGAGTCA TCATCACAGC       1250

CCTGTACCTG AGAGCAAAAT TCAGCAGGAG TGCAGAGACT GCTGCCAACC       1300
```

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```
TGCAGGACCC CAACCAGCTC TACAATGAGC TCAATCTAGG GCGAAGAGAG         1350

GAATATGACG TCTTGGAGAA GAAGCGGGCT CGGGATCCA  AGATGGGAGG         1400

CAAACAGCAG AGGAGGAGGA ACCCCCAGGA AGGCGTATAC AATGCACTGC         1450

AGAAAGACAA GATGCCAGAA GCCTACAGTG AGATCGGCAC AAAAGGCGAG         1500

AGGCGGAGAG GCAAGGGGCA CGATGGCCTT TACCAGGACA GCCACTTCCA         1550

AGCAGTGCAG TTCGGGAACA GAAGAGAGAG AGAAGGTTCA GAACTCACAA         1600

GGACCCTTGG GTTAAGAGCC CGCCCCAAAG GTGAAAGCAC CCAGCAGAGT         1650

AGCCAATCCT GTGCCAGCGT CTTCAGCATC CCCACTCTGT GGAGTCCATG         1700

GCCACCCAGT AGCAGCTCCC AGCTCTAA                                 1728
```

(2) INFORMATION FOR SEQ ID NO:2:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1389 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

```
ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC         50

GCTCCTCCCA GCAGCCACTC AGGGAAACAA AGTGGTGCTG GGCAAAAAAG         100

GGGATACAGT GGAACTGACC TGTACAGCTT CCCAGAAGAA GAGCATACAA         150

TTCCACTGGA AAAACTCCAA CCAGATAAAG ATTCTGGGAA ATCAGGGCTC         200

CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGCT GACTCAAGAA         250

GAAGCCTTTG GGACCAAGGA AACTTCCCCC TGATCATCAA GAATCTTAAG         300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGAGGACC AGAAGGAGGA         350

GGTGCAATTG CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC         400

TTCAGGGGCA GAGCCTGACC CTGACCTTGG AGAGCCCCCC TGGTAGTAGC         450

CCCTCAGTGC AATGTAGGAG TCCAAGGGGT AAAAACATAC AGGGGGGGAA         500

GACCCTCTCC GTGTCTCAGC TGGAGCTCCA GGATAGTGGC ACCTGGACAT         550

GCACTGTCTT GCAGAACCAG AAGAAGGTGG AGTTCAAAAT AGACATCGTG         600
```

```
GTGCTAGCTT TCCAGAAGGC CTCCAGCATA GTCTATAAGA AAGAGGGGGA          650

ACAGGTGGAG TTCTCCTTCC CACTCGCCTT TACAGTTGAA AAGCTGACGG          700

GCAGTGGCGA GCTGTGGTGG CAGGCGGAGA GGGCTTCCTC CTCCAAGTCT          750

TGGATCACCT TTGACCTGAA GAACAAGGAA GTGTCTGTAA AACGGGTTAC          800

CCAGGACCCT AAGCTCCAGA TGGGCAAGAA GCTCCCGCTC CACCTCACCC          850

TGCCCCAGGC CTTGCCTCAG TATGCTGGCT CTGGAAACCT CACCCTGGCC          900

CTTGAAGCGA AAACAGGAAA GTTGCATCAG GAAGTGAACC TGGTGGTGAT          950

GAGAGCCACT CAGCTCCAGA AAAATTTGAC CTGTGAGGTG TGGGGACCCA          1000

CCTCCCCTAA GCTGATGCTG AGCTTGAAAC TGGAGAACAA GGAGGCAAAG          1050

GTCTCGAAGC GGGAGAAGCC GGTGTGGGTG CTGAACCCTG AGGCGGGGAT          1100

GTGGCAGTGT CTGCTGAGTG ACTCGGGACA GGTCCTGCTG GAATCCAACA          1150

TCAAGGTTCT GCCCACATGG TCCACCCCGG TGCACGCGGA TCCGCAGCTC          1200

TGCTATATCC TGGATGCCAT CCTGTTTTTG TATGGTATTG TCCTTACCCT          1250

GCTCTACTGT CGACTCAAGA TCCAGGTCCG AAAGGCAGAC ATAGCCAGCC          1300

GTGAGAAATC AGATGCTGTC TACACGGGCC TGAACACCCG GAACCAGGAG          1350

ACATATGAGA CTCTGAAACA TGAGAAACCA CCCCAATAG                      1389
```

(2) INFORMATION FOR SEQ ID NO:3:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 1599 base pairs
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: double
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: DNA (genomic)

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```
ATGAACCGGG GAGTCCCTTT TAGGCACTTG CTTCTGGTGC TGCAACTGGC          50

GCTCCTCCCA GCAGCCACTC AGGGAAACAA AGTGGTGCTG GGCAAAAAAG          100

GGGATACAGT GGAACTGACC TGTACAGCTT CCCAGAAGAA GAGCATACAA          150

TTCCACTGGA AAAACTCCAA CCAGATAAAG ATTCTGGGAA ATCAGGGCTC          200

CTTCTTAACT AAAGGTCCAT CCAAGCTGAA TGATCGCGCT GACTCAAGAA          250
```

```
GAAGCCTTTG GGACCAAGGA AACTTCCCCC TGATCATCAA GAATCTTAAG        300

ATAGAAGACT CAGATACTTA CATCTGTGAA GTGGAGGACC AGAAGGAGGA        350

GGTGCAATTG CTAGTGTTCG GATTGACTGC CAACTCTGAC ACCCACCTGC        400

TTCAGGGGCA GAGCCTGACC CTGACCTTGG AGAGCCCCCC TGGTAGTAGC        450

CCCTCAGTGC AATGTAGGAG TCCAAGGGGT AAAAACATAC AGGGGGGGAA        500

GACCCTCTCC GTGTCTCAGC TGGAGCTCCA GGATAGTGGC ACCTGGACAT        550

GCACTGTCTT GCAGAACCAG AAGAAGGTGG AGTTCAAAAT AGACATCGTG        600

GTGCTAGCTT TCCAGAAGGC CTCCAGCATA GTCTATAAGA AAGAGGGGGA        650

ACAGGTGGAG TTCTCCTTCC CACTCGCCTT TACAGTTGAA AAGCTGACGG        700

GCAGTGGCGA GCTGTGGTGG CAGGCGGAGA GGGCTTCCTC CTCCAAGTCT        750

TGGATCACCT TTGACCTGAA GAACAAGGAA GTGTCTGTAA AACGGGTTAC        800

CCAGGACCCT AAGCTCCAGA TGGGCAAGAA GCTCCCGCTC CACCTCACCC        850

TGCCCCAGGC CTTGCCTCAG TATGCTGGCT CTGGAAACCT CACCCTGGCC        900

CTTGAAGCGA AAACAGGAAA GTTGCATCAG GAAGTGAACC TGGTGGTGAT        950

GAGAGCCACT CAGCTCCAGA AAAATTTGAC CTGTGAGGTG TGGGGACCCA       1000

CCTCCCCTAA GCTGATGCTG AGCTTGAAAC TGGAGAACAA GGAGGCAAAG       1050

GTCTCGAAGC GGGAGAAGCC GGTGTGGGTG CTGAACCCTG AGGCGGGGAT       1100

GTGGCAGTGT CTGCTGAGTG ACTCGGGACA GGTCCTGCTG GAATCCAACA       1150

TCAAGGTTCT GCCCACATGG TCCACCCCGG TGCACGCGGA TCCCAAACTC       1200

TGCTACCTGC TGGATGGAAT CCTCTTCATC TATGGTGTCA TTCTCACTGC       1250

CTTGTTCCTG AGAGTGAAGT TCAGCAGGAG CGCAGAGCCC CCCGCGTACC       1300

AGCAGGGCCA GAACCAGCTC TATAACGAGC TCAATCTAGG ACGAAGAGAG       1350

GAGTACGATG TTTTGGACAA GAGACGTGGC CGGGACCCTG AGATGGGGGG       1400

AAAGCCGAGA AGGAAGAACC CTCAGGAAGG CCTGTACAAT GAACTGCAGA       1450

AAGATAAGAT GGCGGAGGCC TACAGTGAGA TTGGGATGAA AGGCGAGCGC       1500

CGGAGGGGCA AGGGGCACGA TGGCCTTTAC CAGGGTCTCA GTACAGCCAC       1550

CAAGGACACC TACGACGCCC TTCACATGCA GGCCCTGCCC CCTCGCTAA       1599
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
    (A) LENGTH: 575 amino acids
    (B) TYPE: amin acid
    (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
1               5                   10                  15
Ala Leu Leu Pro Ala Ala Thr Gln Gly Asn Lys Val Val Leu Gly Lys
            20                  25                  30
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
            35                  40                  45
Ile Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Asn
    50                  55                  60
Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala
65                  70                  75                  80
Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Pro Leu Ile Ile
            85                  90                  95
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100                 105                 110
Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115                 120                 125
Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                 135                 140
Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145                 150                 155                 160
Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                 170                 175
Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
            180                 185                 190
Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                 200                 205
Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                 215                 220
Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                 230                 235                 240
Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
                245                 250                 255
Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
            260                 265                 270
Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
            275                 280                 285
Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
    290                 295                 300
Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305                 310                 315                 320
Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
                325                 330                 335
Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
            340                 345                 350
Lys Arg Glu Lys Pro Val Trp Val Leu Asn Pro Glu Ala ly Met Trp
            355                 360                 365
```

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```
Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
    370               375               380
Lys Val Leu Pro Thr Trp Ser Thr Pro Val His Ala Asp Pro Lys Leu
385               390               395               400
Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Ile Thr
            405               410               415
Ala Leu Tyr Leu Arg Ala Lys Phe Ser Arg Ser Ala Glu Thr Ala Ala
            420               425               430
Asn Leu Gln Asp Pro Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
        435               440               445
Arg Glu Glu Tyr Asp Val Leu Glu Lys Lys Arg Ala Arg Asp Pro Glu
    450               455               460
Met Gly Gly Lys Gln Gln Arg Arg Arg Asn Pro Gln Glu Gly Val Tyr
465               470               475               480
Asn Ala Leu Gln Lys Asp Lys Met Pro Glu Ala Tyr Ser Glu Ile Gly
            ·485               490               495
Thr Lys Gly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln
            500               505               510
Asp Ser His Phe Gln Ala Val Gln Phe Gly Asn Arg Arg Glu Arg Glu
        515               520               525
Gly Ser Glu Leu Thr Arg Thr Leu Gly Leu Arg Ala Arg Pro Lys Gly
        530               535               540 ·
Glu Ser Thr Gln Gln Ser Ser Gln Ser Cys Ala Ser Val Phe Ser Ile
555               550               565               560
Pro Thr Leu Trp Ser Pro Trp Pro Pro Ser Ser Ser Ser Gln Leu
            565               570               575
```

(2) INFORMATION FOR SEQ ID NO:5:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 462 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
1               5               10               15
Ala Leu Leu Pro Ala Ala Thr Gln Gly Asn Lys Val Val Leu Gly Lys
            20               25               30
Lys Gly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
        35               40               45
Ile Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Asn
    50               55               60
Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala
65               70               75               80
Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Pro Leu Ile Ile
            85               90               95
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
            100               105               110
Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
        115               120               125
Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
```

```
        130                    135                    140
Ser Pro Pro Gly Ser Ser Pr  Ser Val Gln Cys Arg Ser Pro Arg Gly
145                    150                 -   155                160
Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                    170                    175
Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
                180                    185                    190
Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
                195                    200                    205
Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
210                    215                    220
Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                    230                    235                240
Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
                245                    250                    255
Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
                260                    265                    270
Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
                275                    280                    285
Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
290                    295                    300
Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305                    310                    315                320
Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
                325                    330                  .  335
Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
                340                    345                    350
Lys Arg Glu Lys Pro Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
                355                    360                    365
Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
370                    375                    380
Lys Val Leu Pro Thr Trp Ser Thr Pro Val His Ala Asp Pro Gln Leu
385                    390                    395                400
Cys Tyr Ile Leu Asp Ala Ile Leu Phe Leu Tyr Gly Ile Val Leu Thr
                405                    410                    415
Leu Leu Tyr Cys Arg Leu Lys Ile Gln Val Arg Lys Ala Asp Ile Ala
                420                    425                    430
Ser Arg Glu Lys Ser Asp Ala Val Tyr Thr Gly Leu Asn Thr Arg Asn
                435                    440                    445
Gln Glu Thr Tyr Glu Thr Leu Lys His Glu Lys Pro Pro Gln
450                    455                    460     462
```

(2) INFORMATION FOR SEQ ID NO:6:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 532 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
Met Asn Arg Gly Val Pro Phe Arg His Leu Leu Leu Val Leu Gln Leu
1               5               10                      15
```

```
Ala Leu Leu Pro Ala Ala Thr Gln Gly Asn Lys Val Val Leu Gly Lys
        20                      25                      30
Lys  ly Asp Thr Val Glu Leu Thr Cys Thr Ala Ser Gln Lys Lys Ser
        35                      40                      45
Ile Gln Phe His Trp Lys Asn Ser Asn Gln Ile Lys Ile Leu Gly Asn
        50                      55                      60
Gln Gly Ser Phe Leu Thr Lys Gly Pro Ser Lys Leu Asn Asp Arg Ala
65                  70                      75                  80
Asp Ser Arg Arg Ser Leu Trp Asp Gln Gly Asn Phe Pro Leu Ile Ile
                    85                  90                      95
Lys Asn Leu Lys Ile Glu Asp Ser Asp Thr Tyr Ile Cys Glu Val Glu
                100                     105                     110
Asp Gln Lys Glu Glu Val Gln Leu Leu Val Phe Gly Leu Thr Ala Asn
            115                     120                     125
Ser Asp Thr His Leu Leu Gln Gly Gln Ser Leu Thr Leu Thr Leu Glu
    130                     135                     140
Ser Pro Pro Gly Ser Ser Pro Ser Val Gln Cys Arg Ser Pro Arg Gly
145                     150                     155                 160
Lys Asn Ile Gln Gly Gly Lys Thr Leu Ser Val Ser Gln Leu Glu Leu
                165                     170                     175
Gln Asp Ser Gly Thr Trp Thr Cys Thr Val Leu Gln Asn Gln Lys Lys
                180                     185                     190
Val Glu Phe Lys Ile Asp Ile Val Val Leu Ala Phe Gln Lys Ala Ser
        195                     200                     205
Ser Ile Val Tyr Lys Lys Glu Gly Glu Gln Val Glu Phe Ser Phe Pro
    210                     215                     220
Leu Ala Phe Thr Val Glu Lys Leu Thr Gly Ser Gly Glu Leu Trp Trp
225                     230                     235                 240
Gln Ala Glu Arg Ala Ser Ser Ser Lys Ser Trp Ile Thr Phe Asp Leu
                245                     250                     255
Lys Asn Lys Glu Val Ser Val Lys Arg Val Thr Gln Asp Pro Lys Leu
                260                     265                     270
Gln Met Gly Lys Lys Leu Pro Leu His Leu Thr Leu Pro Gln Ala Leu
        275                     280                     285
Pro Gln Tyr Ala Gly Ser Gly Asn Leu Thr Leu Ala Leu Glu Ala Lys
        290                     295                     300
Thr Gly Lys Leu His Gln Glu Val Asn Leu Val Val Met Arg Ala Thr
305                     310                     315                 320
Gln Leu Gln Lys Asn Leu Thr Cys Glu Val Trp Gly Pro Thr Ser Pro
                325                     330                     335
Lys Leu Met Leu Ser Leu Lys Leu Glu Asn Lys Glu Ala Lys Val Ser
        340                     345                     350
Lys Arg Glu Lys Pro Val Trp Val Leu Asn Pro Glu Ala Gly Met Trp
        355                     360                     365
Gln Cys Leu Leu Ser Asp Ser Gly Gln Val Leu Leu Glu Ser Asn Ile
    370                     375                     380
Lys Val Leu Pro Thr Trp Ser Thr Pro Val His Ala Asp Pro Lys Leu
385                     390                     395                 400
Cys Tyr Leu Leu Asp Gly Ile Leu Phe Ile Tyr Gly Val Ile Leu Thr
                405                     410                     415
Ala Leu Phe Leu Arg Val Lys Phe Ser Arg Ser Ala Glu Pro Pro Ala
                420                     425                     430
Tyr Gln Gln Gly Gln Asn Gln Leu Tyr Asn Glu Leu Asn Leu Gly Arg
            435                     440                     445
Arg Glu Glu Tyr Asp Val Leu Asp Lys Arg Arg Gly Arg Asp Pro Glu
450                     455                     460
```

```
Met Gly Gly Lys Pro Arg Arg Lys Asn Pro Gln  lu Gly Leu Tyr Asn
465               470             475           480
Glu Leu Gln Lys Asp Lys Met Ala Glu Ala Tyr Ser  lu Ile Gly Met
            485             490             495
Lys _ly Glu Arg Arg Arg Gly Lys Gly His Asp Gly Leu Tyr Gln Gly
        500             505             510
Leu Ser Thr Ala Thr Lys Asp Thr Tyr Asp Ala Leu His Met Gln Ala
        515             520             525
Leu Pro Pro Arg
    530
```

(2) INFORMATION FOR SEQ ID NO:7:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CGCGGGGTGA CCGTGCCCTC CAGCAGCTTG GGC   33

(2) INFORMATION FOR SEQ ID NO:8:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 50 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGCGGGGATC CGTCGTCCAG AGCCCGTCCA GCTCCCCGTC CTGGGCCTCA   50

(2) INFORMATION FOR SEQ ID NO:9:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

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    (xi) SEQUENCE DESCRIPTI N: SEQ ID NO:9:

    CGCGGGCGGC CGCGACGCCG GCCAAGACAG CAC   33

    (2) INFORMATION FOR SEQ ID NO:10:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 33 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

    CGCGTTGACG AGCAGCCAGT TGGGCAGCAG CAG   33

    (2) INFORMATION FOR SEQ ID NO:11:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 15 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

    CGCGGGCGGC CGCTA   15

    (2) INFORMATION FOR SEQ ID NO:12:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 42 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

    CGCGGGCTCG TTATAGAGCT GGTTCTGGCG CTGCTTCTTC TG   42

    (2) INFORMATION FOR SEQ ID NO:13:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 48 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: nucleic acid

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGCGGGGAGC TCGTTATAGA GCTGGTTTGC CGCCGAATTC TTATCCCG   48

(2) INFORMATION FOR SEQ ID NO:14:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: nucleic acid

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGCGGGGCGG CCACGCGTCC TCGCCAGCAC ACA   33

(2) INFORMATION FOR SEQ ID NO:15:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 36 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: nucleic acid

  (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGGACGC GTTTCAGCCG TCCTCGCCAG CACACA   36

(2) INFORMATION FOR SEQ ID NO:16:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

  (ii) MOLECULE TYPE: nucleic acid

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(xi) SEQUENCE DESCRIPTI N: SEQ ID N :16:

CGCGGGACGC GTGACCCTGA GATGGGGGGA AA   33

(2) INFORMATION FOR SEQ ID NO:17:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

CGCGGGACGC GTATTGGGAT GAAAGGCGAG CGC   33

(2) INFORMATION FOR SEQ ID NO:18:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 26 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CCCGGATCCC AGCATGGGCA GCTCTT   26

(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 42 bases
        (B) TYPE: nucleic acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: nucleic acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

CGCGGGGCGG CCGCTTTAGT TATTACTGTT GACATGGTCG TT   42

(2) INFORMATION FOR SEQ ID NO:20:

    (i) SEQUENCE CHARACTERISTICS:

            (A) LENGTH: 30 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

GCGGGGGGAT CCCACTGTCC AAGCTCCCAG   30

(2) INFORMATION FOR SEQ ID NO:21:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 32 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

GCGGGGGCGG CCGCCTAAAT ACGGTTCTGG TC   32

(2) INFORMATION FOR SEQ ID NO:22:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 31 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TCAGAAAGAG ACAACCTGAA GAAACCAACA A   31

(2) INFORMATION FOR SEQ ID NO:23:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 31 bases
            (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: nucleic acid

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(xi) SEQUENCE DESCRIPTI N: SEQ ID N :23:

TTGTTGGTTT CTTCAGGTTG TGTCTTTCTG A   31

(2) INFORMATION FOR SEQ ID NO:24:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 171 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: amino acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

Met Glu His Ser Thr Phe Leu Ser Gly Leu Val Leu Ala Thr Leu Leu
                5   10   15
Ser Gln Val Ser Pro Phe Lys Ile Pro Ile Glu Glu Leu Glu Asp Arg
            20   25   30
Val Phe Val Asn Cys Asn Thr Ser Ile Thr Trp Val Glu Gly Thr Val
        35   40   45
Gly Thr Leu Leu Ser Asp Ile Thr Arg Leu Asp Leu Gly Lys Arg Ile
    50   55   60
Leu Asp Pro Arg Gly Ile Tyr Arg Cys Asn Gly Thr Asp Ile Tyr Lys
65   70   75   80
Asp Lys Glu Ser Thr Val Gln Val His Tyr Arg Met Cys Gln Ser Cys
                85   90   95
Val Glu Leu Asp Pro Ala Thr Val Ala Gly Ile Ile Val Thr Asp Val
                100   105   110
Ala Ile Thr Leu Leu Leu Ala Leu Gly Val Phe Cys Phe Ala Gly His
    115   120   125
Glu Thr Gly Arg Leu Ser Gly Ala Ala Asp Thr Gln Ala Leu Leu Arg
    130   135   140
Asn Asp Gln Val Tyr Gln Pro Leu Arg Asp Arg Asp Asp Ala Gln Tyr
145   150   155   160
Ser His Leu Gly Gly Asn Trp Ala Arg Asn Lys
            165   170

(2) INFORMATION FOR SEQ ID NO:25:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 182 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: amino acid

    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Met Glu Gln Gly Lys Gly Leu Ala Val Leu Ile Leu Ala Ile Ile Leu
                5   10   15

```
Leu Gln Gly Thr Leu Ala Gln Ser Ile Lys Gly Asn His Leu Val Lys
             20                  25                  30
Val Tyr Asp Tyr Gln Glu Asp Gly Ser Val Leu Leu Thr Cys Asp Ala
         35                  40                  45
Glu Ala Lys Asn Ile Thr Trp Phe Lys Asp Gly Lys Met Ile Gly Phe
     50                  55                  60
Leu Thr Glu Asp Lys Lys Lys Trp Asn Leu Gly Ser Asn Ala Lys Asp
65                  70                  75                  80
Pro Arg Gly Met Tyr Gln Cys Lys Gly Ser Gln Asn Lys Ser Lys Pro
                 85                  90                  95
Leu Gln Val Tyr Tyr Arg Met Cys Gln Asn Cys Ile Glu Leu Asn Ala
                100                 105                 110
Ala Thr Ile Ser Gly Phe Leu Phe Ala Glu Ile Val Ser Ile Phe Val
         115                 120                 125
Leu Ala Val Gly Val Tyr Phe Ile Ala Gly Gln Asp Gly Val Arg Gln
         130                 135                 140
Ser Arg Ala Ser Asp Lys Gln Thr Leu Leu Pro Asn Asp Gln Leu Tyr
145                 150                 155                 160
Gln Pro Leu Lys Asp Arg Glu Asp Asp Gln Tyr Ser His Leu Gln Gly
                 165                 170                 175
Asn Gln Leu Arg Arg Asn
             180
```

(2) INFORMATION FOR SEQ ID NO:26:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 220 amino acids
            (B) TYPE: amino acid
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: amino acids

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

```
Met Pro Gly Gly Leu Glu Ala Leu Arg Ala Leu Pro Leu Leu Leu Phe
                 5                  10                  15
Leu Ser Tyr Ala Cys Leu Gly Pro Gly Cys Gln Ala Leu Arg Val Glu
             20                  25                  30
Gly Gly Pro Pro Ser Leu Thr Val Asn Leu Gly Glu Glu Ala Arg Leu
         35                  40                  45
Thr Cys Glu Asn Asn Gly Arg Asn Pro Asn Ile Thr Trp Trp Phe Ser
     50                  55                  60
Leu Gln Ser Asn Ile Thr Trp Pro Pro Val Pro Leu Gly Pro Gly Gln
65                  70                  75                  80
Gly Thr Thr Gly Gln Leu Phe Phe Pro Glu Val Asn Lys Asn Thr Gly
                 85                  90                  95
Ala Cys Thr Gly Cys Gln Val Ile Glu Asn Asn Ile Leu Lys Arg Ser
                100                 105                 110
Cys Gly Thr Tyr Leu Arg Val Arg Asn Pro Val Pro Arg Pro Phe Leu
         115                 120                 125
Asp Met Gly Glu Gly Thr Lys Asn Arg Ile Ile Thr Ala Glu Gly Ile
         130                 135                 140
Ile Leu Leu Phe Cys Ala Val Val Pro Gly Thr Leu Leu Leu Phe Arg
145                 150                 155                 160
```

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```
Lys Arg Trp Gln Asn Glu Lys Phe Gly Val Asp Met Pro Asp Asp Tyr
              165                 170                 175
 lu Asp Glu Asn Leu Tyr Glu Gly Leu Asn Leu Asp Asp Cys Ser Met
              180                 185                 190
Tyr Glu Asp Il  Ser Arg Gly Leu ln  Gly Thr Tyr ln  Asp Val Gly
              195                 200                 205
Asn Leu His Ile Gly Asp Ala Gln Leu Glu Lys Pro
    210                 215                 220
```


(2) INFORMATION FOR SEQ ID NO:27:

   (i) SEQUENCE CHARACTERISTICS:
       (A) LENGTH: 228 amino acids
       (B) TYPE: amino acid
       (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: amino acid

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:


```
Met Ala Thr Leu Val Leu Ser Ser Met Pro Cys His Trp Leu Leu Phe
              5                 10                 15
Leu Leu Leu Leu Phe Ser Gly Glu Pro Val Pro Ala Met Thr Ser Ser
              20                25                  30
Asp Leu Pro Leu Asn Phe Gln Gly Ser Pro Cys Ser Gln Ile Trp Gln
              35                40                  45
His Pro Arg Phe Ala Ala Lys Lys Arg Ser Ser Met Val Lys Phe His
    50                55                  60
Cys Tyr Thr Asn His Ser Gly Ala Leu Thr Trp Phe Arg Lys Arg Gly
65                  70                  75                  80
Ser Gln Gln Pro Gln Glu Leu Val Ser Glu Glu Gly Arg Ile Val Gln
              85                90                  95
Thr Gln Asn Gly Ser Val Tyr Thr Leu Thr Ile Gln Asn Ile Gln Tyr
              100               105                 110
Glu Asp Asn Gly Ile Tyr Phe Cys Lys Gln Lys Cys Asp Ser Ala Asn
              115               120                 125
His Asn Val Thr Asp Ser Cys Gly Thr Glu Leu Leu Val Leu Gly Phe
    130               135                 140
Ser Thr Leu Asp Gln Leu Lys Arg Arg Asn Thr Leu Lys Asp Gly Ile
145               150                 155                 160
Ile Leu Ile Gln Thr Leu Leu Ile Ile Leu Phe Ile Ile Val Pro Ile
              165               170                 175
Phe Leu Leu Leu Asp Lys Asp Asp Gly Lys Ala Gly Met Glu Glu Asp
              180               185                 190
His Thr Tyr Glu Gly Leu Asn Ile Asp Gln Thr Ala Thr Tyr Glu Asp
              195               200                 205
Ile Val Thr Leu Arg Thr Gly Glu Val Lys Trp Ser Val Gly Glu His
    210               215                 220
Pro Gly Gln Glu
225
```

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Claims

1   1. A method of directing a cellular r sponse to an
2   infective agent, to a cell infected with said agent, to a
3   tumor or cancerous cell, or to an autoimmune-generated cell in
4   a mammal, said method comprising administering to said mammal
5.   an effective amount of therapeutic cells, said cells being
6   capable of specifically recognizing and destroying said agent
7   or said cell.

1   2. The method of claim 1, wherein said cellular response
2   is MHC-independent.

1   3. The method of claim 1, wherein said therapeutic cells
2   express a membrane-bound, proteinaceous chimeric receptor,
3   comprising (a) an extracellular portion which is capable of
4   specifically recognizing and binding said agent or said cell,
5   and (b) an intracellular or transmembrane portion which is
6   capable of signalling said therapeutic cell to destroy a
7   receptor-bound agent or a receptor-bound cell.

1   4. The method of claim 3, wherein said intracellular
2   portion or said transmembrane portion is a signal-transducing
3   portion of a T cell receptor protein, an Fc receptor protein,
4   or a B cell receptor protein, or a functional derivative
5   thereof.

1   5. The method of claim 4, wherein said receptor protein
2   is ς, η, γ, CD3 delta, T3 gamma, mb1, or B29.

1   6. The method of claim 5, wherein said chimeric receptor
2   comprises:
3   (a) amino acids 421-532 of SEQ ID NO: 6;
4   (b) amino acids 423-455, 438-455, 461-494 or 494-528 of
5   SEQ ID NO:6;
6   (c) amino acids 400-420 of SEQ ID NO:6;
7   (d) amino acids 421-575 of SEQ ID NO: 4;

8   ( ) amino acids 423-455, 438-455, 461-494 or 494-528 of

9   SEQ ID NO:4;

10   (f) amino acids 400-420 of SEQ ID NO: 4;

11   (g) amino acids 421-462 of SEQ ID NO: 5;

12   (h) amino acids 402-419 of SEQ ID NO:5;

13   (i) amino acids Tyr282-Tyr298 inclusive of Fig. 15A;

14   (j) amino acids 132-171 of Fig. 16 (SEQ ID NO: 24);

15   (k) amino acids 140-182 of Fig. 17 (SEQ ID NO: 25);

16   (l) amino acids 162-220 of Fig. 18 (SEQ ID NO: 26);

17   (m) amino acids 183-228 of Fig. 19 (SEQ ID NO: 27).

1   7. The method of claim 1, wherein said therapeutic cells

2   are selected from the group consisting of:

3   (a) T lymphocytes;

4   (b) cytotoxic T lymphocytes:

5   (c) natural killer cells;

6   (d) neutrophils;

7   (e) granulocytes;

8   (f) macrophages;

9   (g) mast cells;

10   (h) HeLa cells; and

11   (i) embryonic stem cells (ES).

1   8. The method of claim 1, wherein said infective agent

2   is an immunodeficiency virus.

1   9. The method of claim 8, wherein said extracellular

2   portion comprises an HIV envelope-binding portion of CD4, or a

3   functional HIV envelope-binding derivative thereof.

1   10. The method of claim 9, wherein said cytotoxic T

2   lymphocyte is a CD8$^+$ cytotoxic T lymphocytes.

1   11. A cell which expresses a proteinaceous membrane-

2   bound chimeric receptor, said receptor comprising (a) an

3   extracellular portion which is capable of specifically

4   recognizing and binding an infective agent, a cell infected

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5   with an infective agent, a tumor or cancerous cell, or an
6   autoimmune-generated cell, and (b) an intracellular portion or
7   transmembrane portion derived from a T cell receptor, an Fc
8   receptor, or a B cell receptor which is capable of signalling
9   said cell to destroy a receptor-bound agent or receptor-bound
10   cell.

1   12.   The cell of claim 11, wherein said binding is MHC-
2   independent.

1   13.   The cell of claim 11, wherein said receptor protein
2   is ʃ, η, γ, CD3 delta, T3 gamma, mb1, or B29.

1   14.   The cell of claim 13, wherein said chimeric receptor
2   comprises amino acids:.
3   (a) amino acids 421-532 of SEQ ID NO: 6;
4   (b) amino acids 423-455, 438-455, 461-494 or 494-528 of
5   SEQ ID NO:6;
6   (c) amino acids 400-420 of SEQ ID NO:6;
7   (d) amino acids 421-575 of SEQ ID NO: 4;
8   (e) amino acids 423-455, 438-455, 461-494 or 494-528 of
9   SEQ ID NO:4;
10   (f) amino acids 400-420 of SEQ ID NO: 4;
11   (g) amino acids 421-462 of SEQ ID NO: 5;
12   (h) amino acids 402-419 of SEQ ID NO:5;
13   (i) amino acids Tyr282-Tyr298 inclusive of Fig. 15A;
14   (j) amino acids 132-171 of Fig. 16 (SEQ ID NO: 24);
15   (k) amino acids 140-182 of Fig. 17 (SEQ ID NO: 25);
16   (l) amino acids 162-220 of Fig. 18 (SEQ ID NO: 26);
17   (m) amino acids 183-228 of Fig. 19 (SEQ ID NO: 27).

1   15.   The cell of claim 11, wherein said therapeutic cells
2   are selected from the group consisting of:
3   (a)   T lymphocytes;
4   (b)   cytotoxic T lymphocytes:
5   (c)   natural killer cells;
6   (d)   neutrophils;

7   ( )   granulocytes;

8   (f)   macrophages;

9   (g)   mast cells;

10   (h)   HeLa cells; and

11   (i)   embryonic stem cells (ES).

1   16.   The cell of claim 11, wherein said extracellular

2   portion comprises an HIV envelope-binding portion of CD4, or a

3   functional HIV envelope-binding derivative thereof.

1   17.   The cell of claim 16, wherein said cytotoxic T

2   lymphocyte is a CD8$^+$ cytotoxic T lymphocytes.

1   18.   The cell of claim 11, wherein said extracellular

2   portion is an immunoglobulin molecule, or an antigen-binding

3   fragment thereof.

1   19.   DNA encoding a chimeric receptor of claim 11.

1   20.   A vector comprising the chimeric receptor DNA of

2   claim 19.

1   21.   A method of treating an immunodeficiency virus

2   infection, comprising administering the host cell of claim 16

3   to a mammal.

1   22.   A substantially pure antibody which specifically

2   recognizes and binds a chimeric receptor of claim 11.
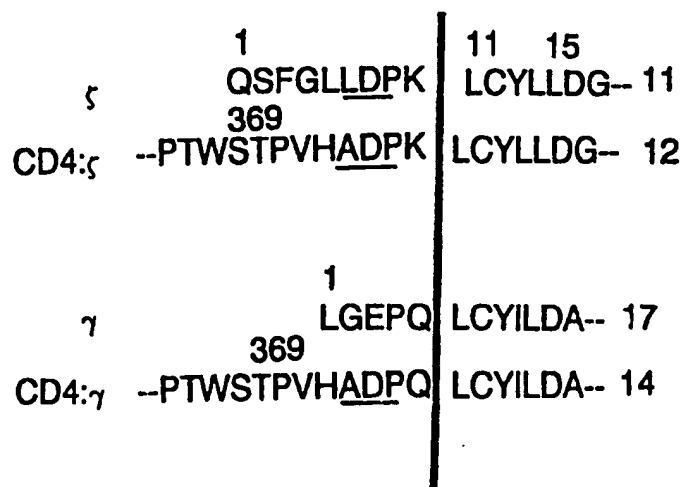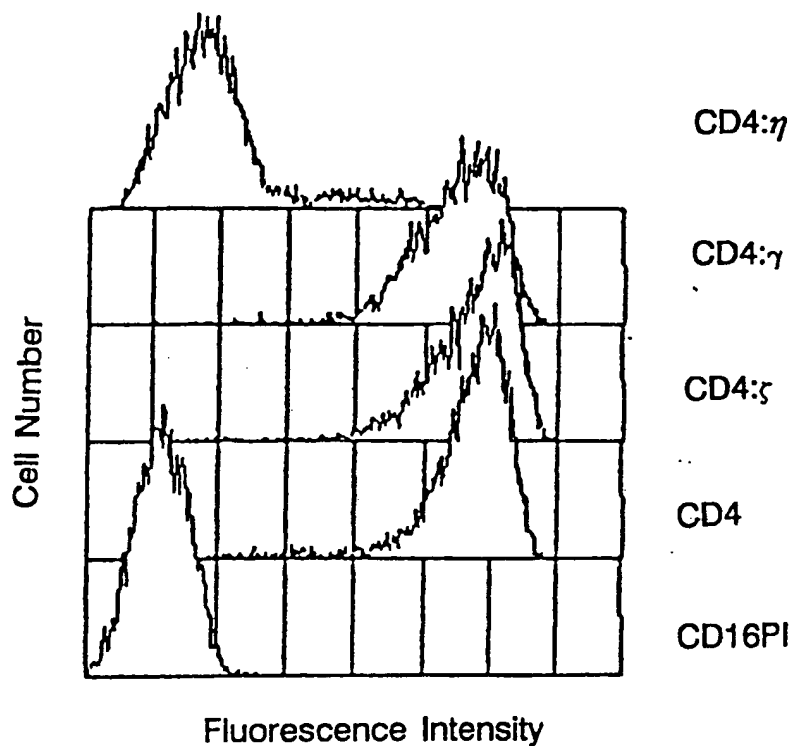
```
              1                    11   15
            QSFGLLDPK         │ LCYLLDG-- 11
  ς         369
 CD4:ς     --PTWSTPVHADPK     │ LCYLLDG-- 12


                     1
                    LGEPQ     │ LCYILDA-- 17
  γ         369
 CD4:γ     --PTWSTPVHADPQ     │ LCYILDA-- 14
```

FIG. 1a

CD4:η

CD4:γ

CD4:ς

CD4

CD16PI

Cell Number

Fluorescence Intensity

FIG. 1b

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FIG. 1c

FIG. 2

FIG. 3a

FIG. 3b

FIG. 4a

Time in Seconds

FIG. 4b

Time in Seconds

FIG. 4c

Time In Seconds

FIG. 4d

Time In Seconds

FIG. 5a

E/T Ratio

FIG. 5b

E/T Ratio

FIG. 5c

CD4:ς   60% Pos..

CD4:γ   13% Pos.

CD4:η   60% Pos.

CD4:ς   C11G/D15G   38% Pos.

Uninfected   4% Pos. .

FIG. 6a

FIG. 6b

FIG. 7a

CD16

CD16:ζ

1
QSFGLLDPKLCYLLDG   ζ

189
--FSPPGADPKLCYLLDG   CD16:ζ

FIG. 7b

■ Jurkat, OKT3

▲ REX 33A, CD16:ζ

□ JRT3.T3.5, CD16:ζ

△ Jurkat, CD16:ζ

+ Jurkat, CD16

• REX 33A, CD16

FIG. 8a

```
                                         F34*                        Y51*
1   QSFGLLDPKL  CYLLDGILFI  YGVILTALFL  RVKFSRSAEP  PAYQQGQNQL
                                           ←┘                          ←┘

          E60*    D66*
51  YNELNLGRRE  EYDVLDKRRG  RDPEMGGKPRRKNPQEGLYN  ELQKDKMAEA
          ←┘      ←┘

                           G122*        A133*       L139*
101 YSEIGMKGER  RRGKGHDGLY  QGLSTATKDT  YDALHMQALP  PR
                             ←┘          ←┘     ←┘
```

FIG. 8b

FIG. 9a

| CD16:ζC11G/D15G/D66* |
| CD16:5ζ |
| CD16:5:ζ(48-65) |
| CD16:7 |
| CD16:7:ζ(48-65)) |

RVKFSRSAEPPAYQQGQ NQLYNELNLGRREEYDVL

CD16:5:ζ(48-65)   KKLVKKFRQKKQRQ NQLYNELNLGRREEYDVL

CD16:7:ζ(48-65)   RTQIKKLCSWRDKNSAA NQLYNELNLGRREEYDVL

FIG. 9b

Legend:
- CD16:ζ
- CD16:ζD66*
- CD16:ζD66* C11G/D15G
- CD16:ζ E60* C11G/D15G

X-axis: E/T Ratio
Y-axis: % Chromium Released

FIG. 9c

FIG. 9d

FIG. 10a

FIG. 10b

FIG. 10c

- ■ CD16:7:ζ(48-65)
- □   "   E60Q
- +   "   D63N
- ▲   "   Y62F
- o   "   E61Q
- △   "   Y62S

Time in Seconds

FIG. 10f

- ■ CD16:7:ζ(48-65)
- ▲   "   N48S
- □   "   L50S
- △   "   Y51F

Time in Seconds
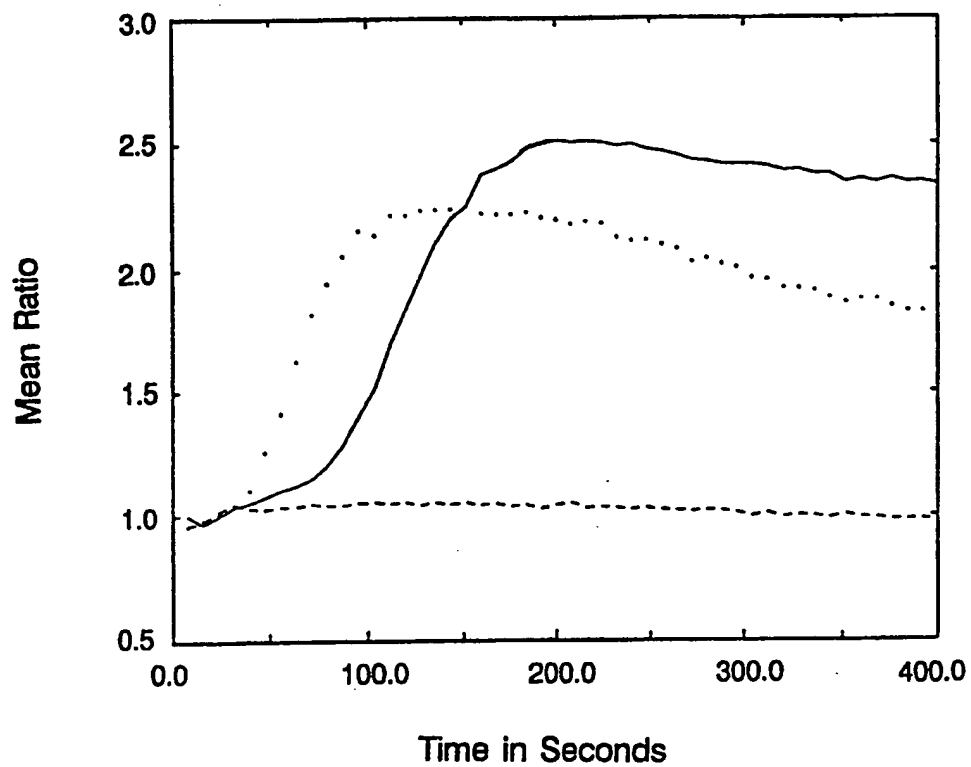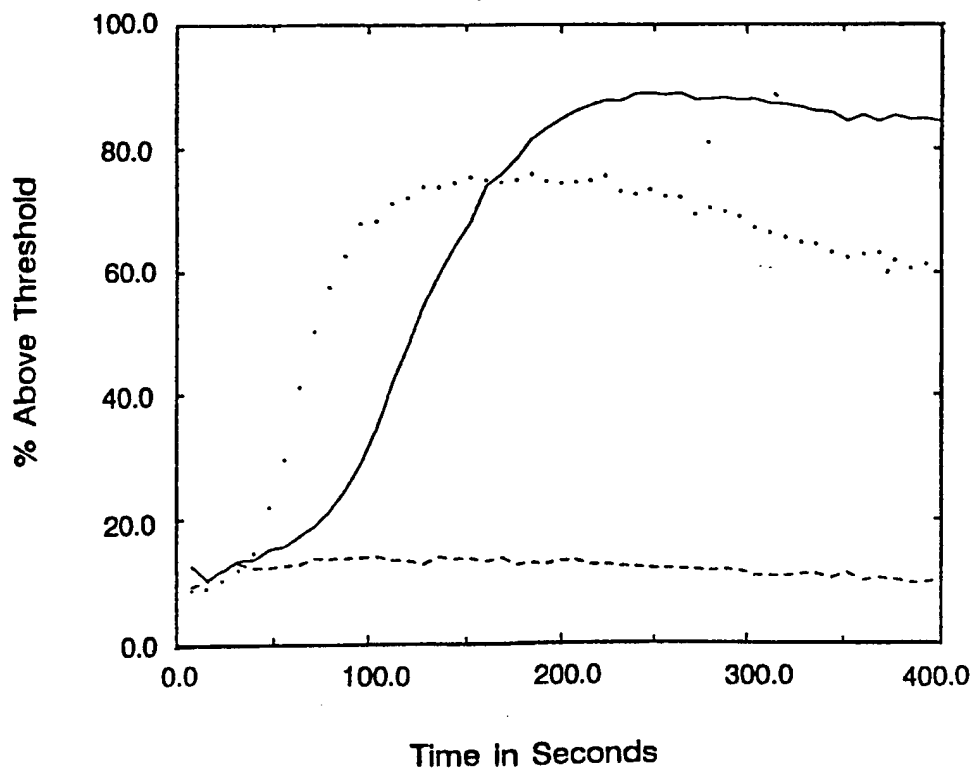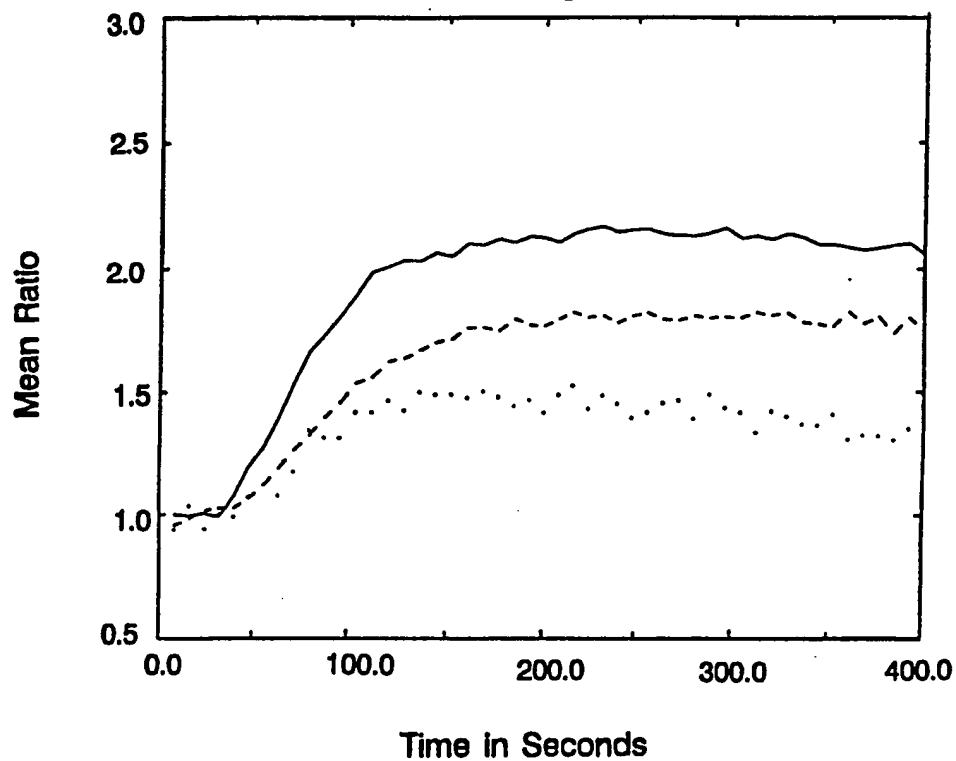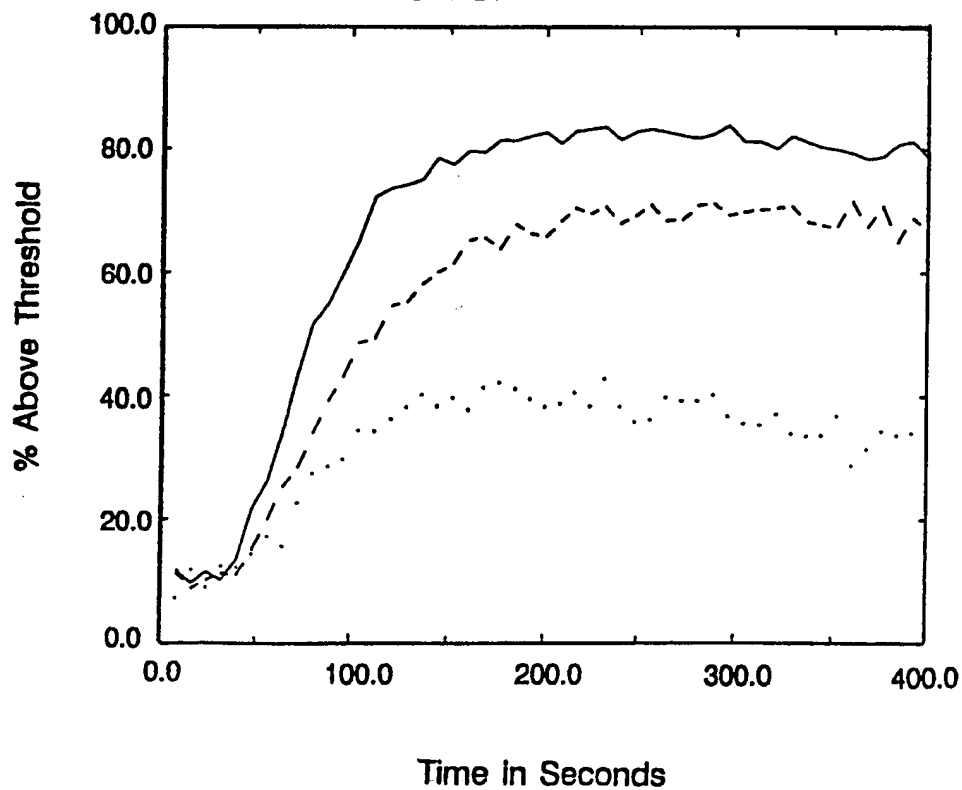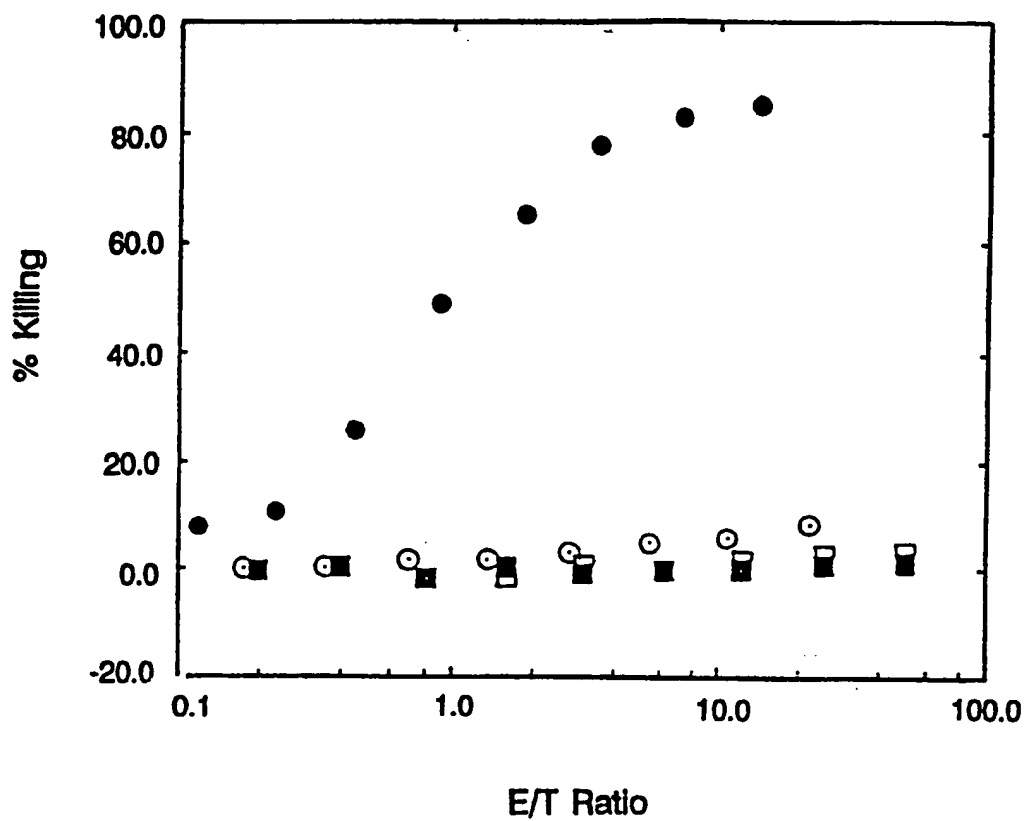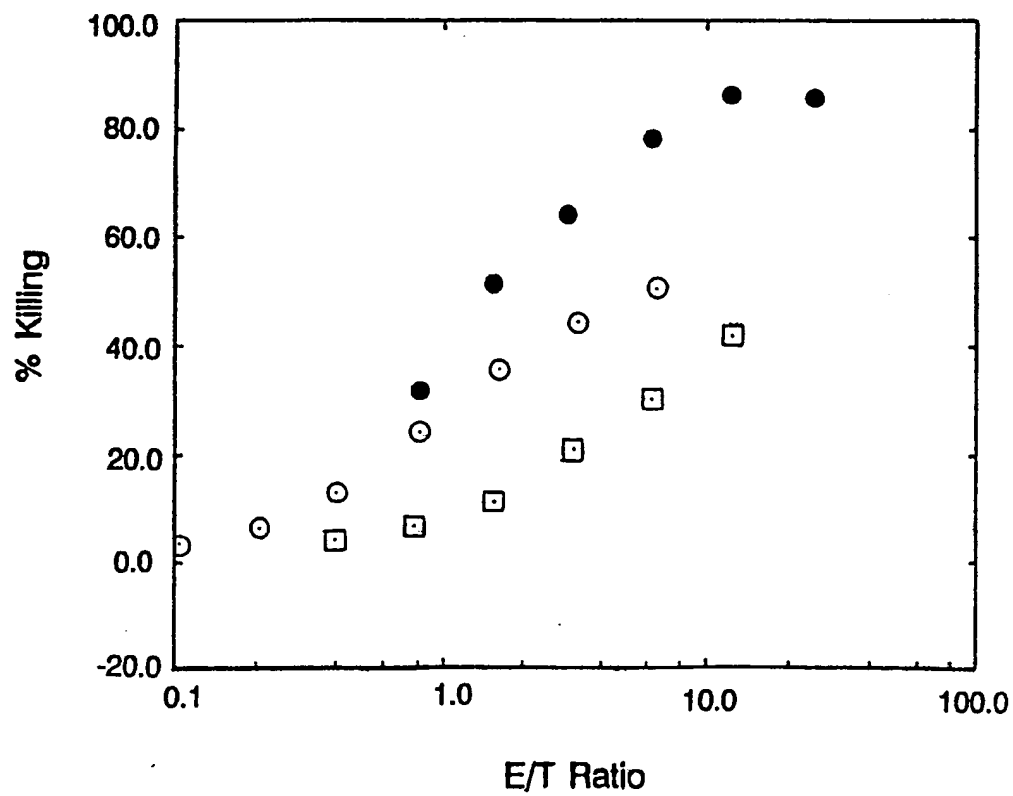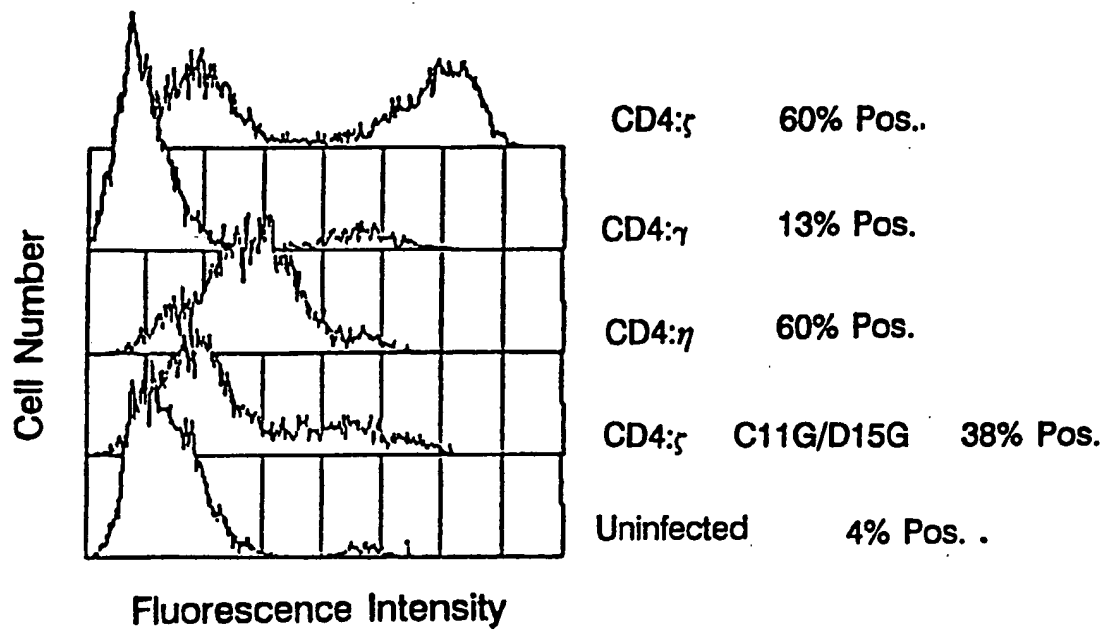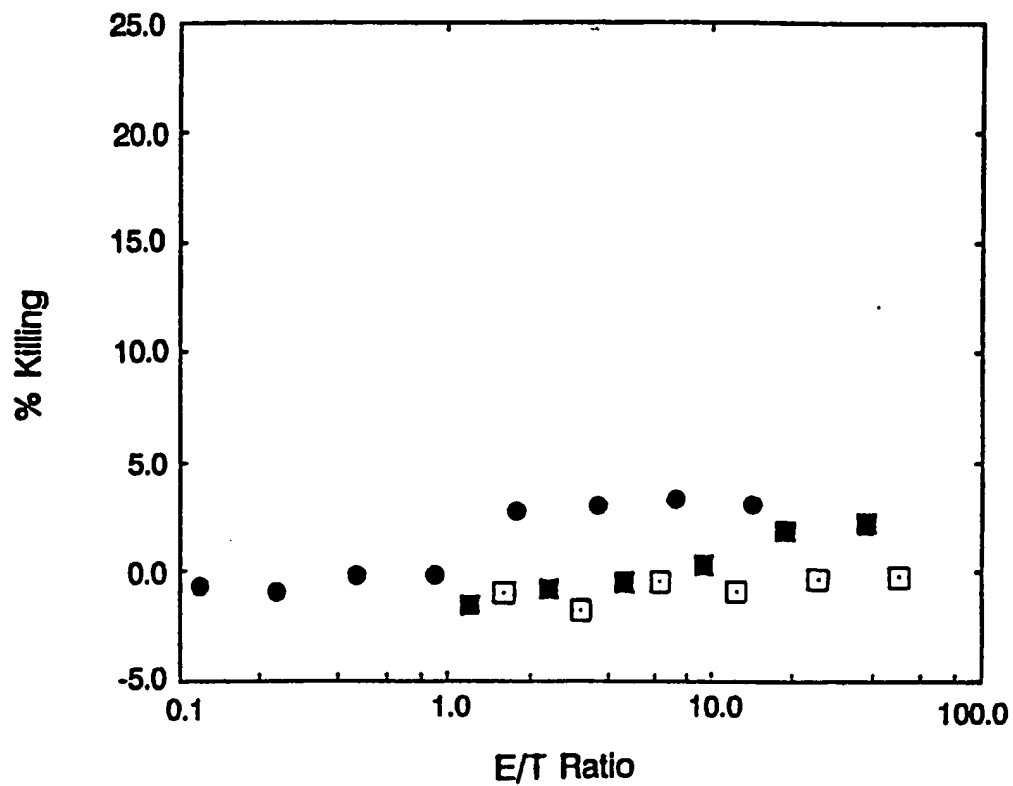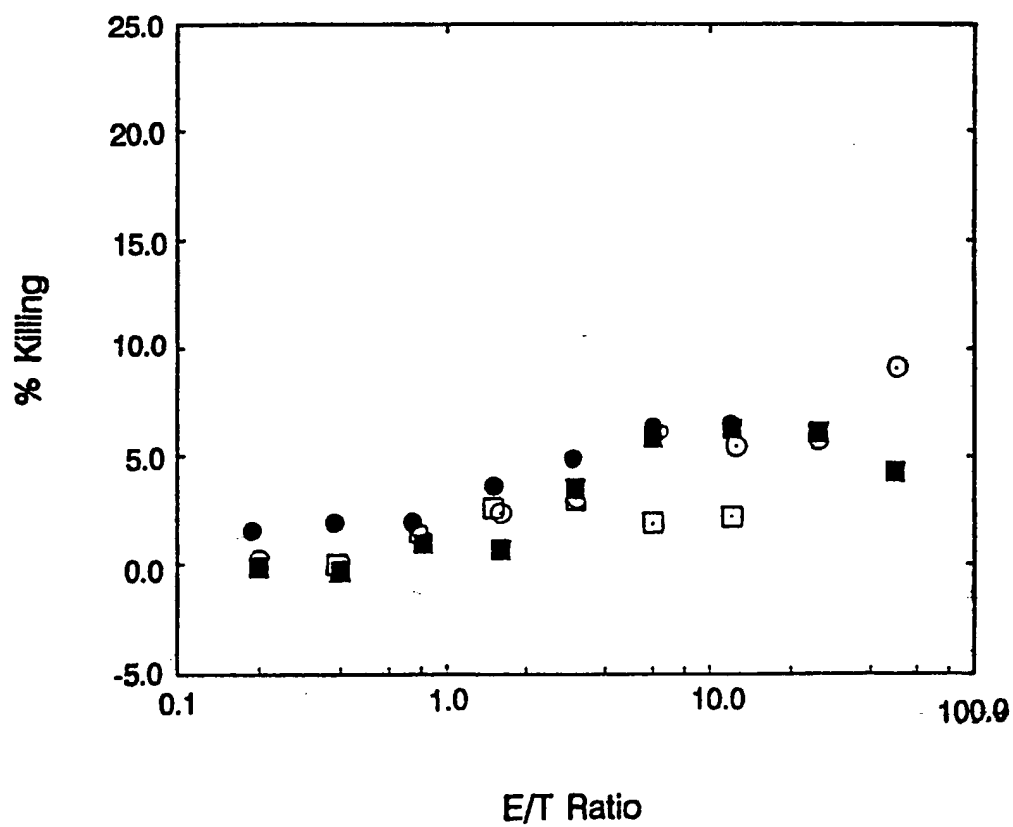
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FIG. 10d

FIG. 10e

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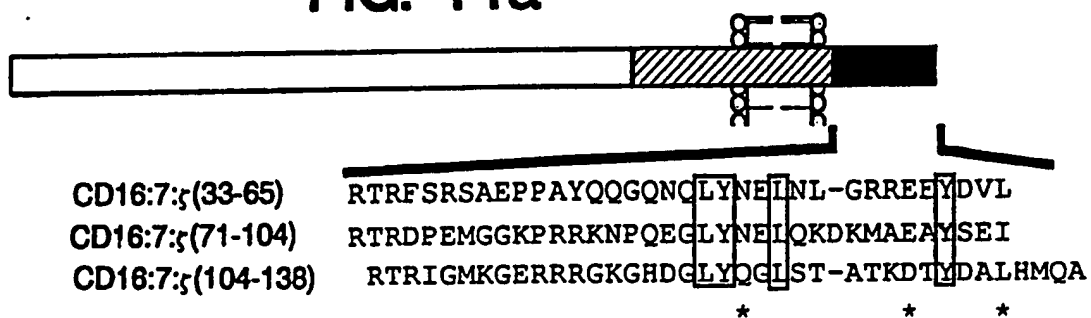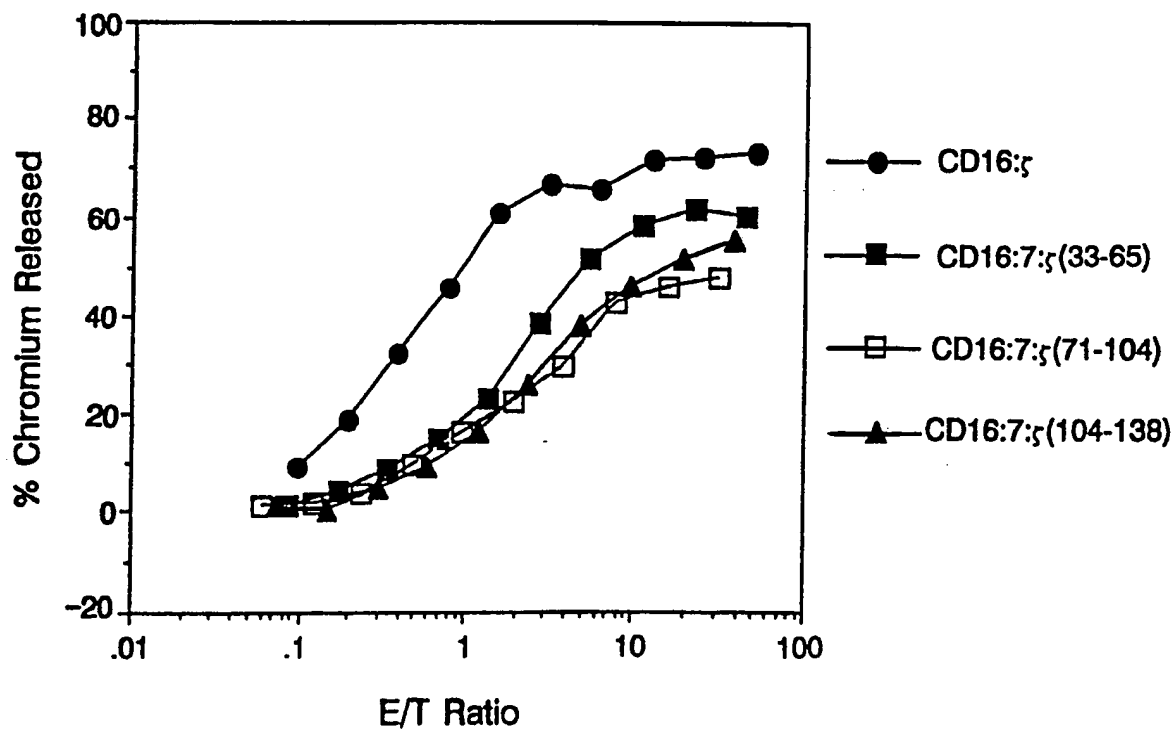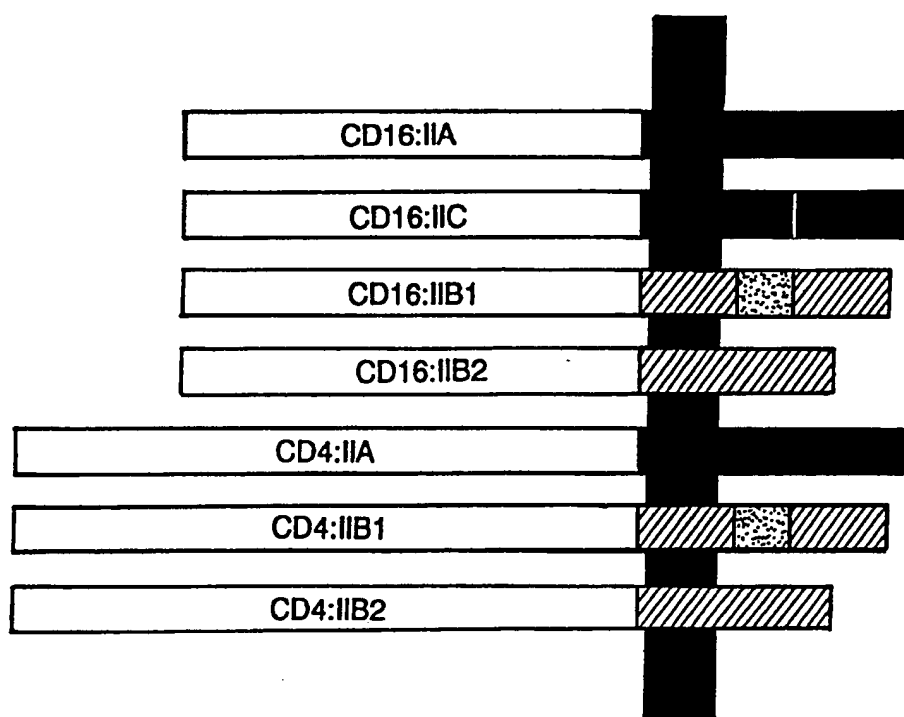
FIG. 11a

CD16:7:ς(33-65)   RTRFSRSAEPPAYQQGQNCLYNELNL-GRREEYDVL
CD16:7:ς(71-104)   RTRDPEMGGKPRRKNPQEGLYNELQKDKMAEAYSEI
CD16:7:ς(104-138)   RTRIGMKGERRRGKGHDGLYQGLST-ATKDTYDALHMQA
                                      *   *   *

FIG. 11b

y-axis: % Chromium Released
x-axis: E/T Ratio

- CD16:ς
- CD16:7:ς(33-65)
- CD16:7:ς(71-104)
- CD16:7:ς(104-138)

FIG. 12

FIG. 13a

FIG. 13b

Time in Seconds   **SUBSTITUTE SHEET**

FIG. 14a

FIG. 14b

FIG. 15a

(269-311)

236 RKKRISANST DPVKAAQFEP PGRQMIAIRK RQLEETNNDY

(276-311)   (282-311)   (299-311)

276 ETADGGYMTL NPRAPTDDDK NIYLTLPPND HVNSNN

Y282*   Y298*

FIG. 15b

■ CD16:7:IIA

△ CD16:IIA(Y298*)

▲ CD16:IIA(Y282*)

Mean Ratio

Time in Seconds

FIG. 15c

● CD16:IIA
■ CD16:7:IIA
△ CD16:IIA298*
▲ CD16:IIA282*

% Killing

E/T Ratio
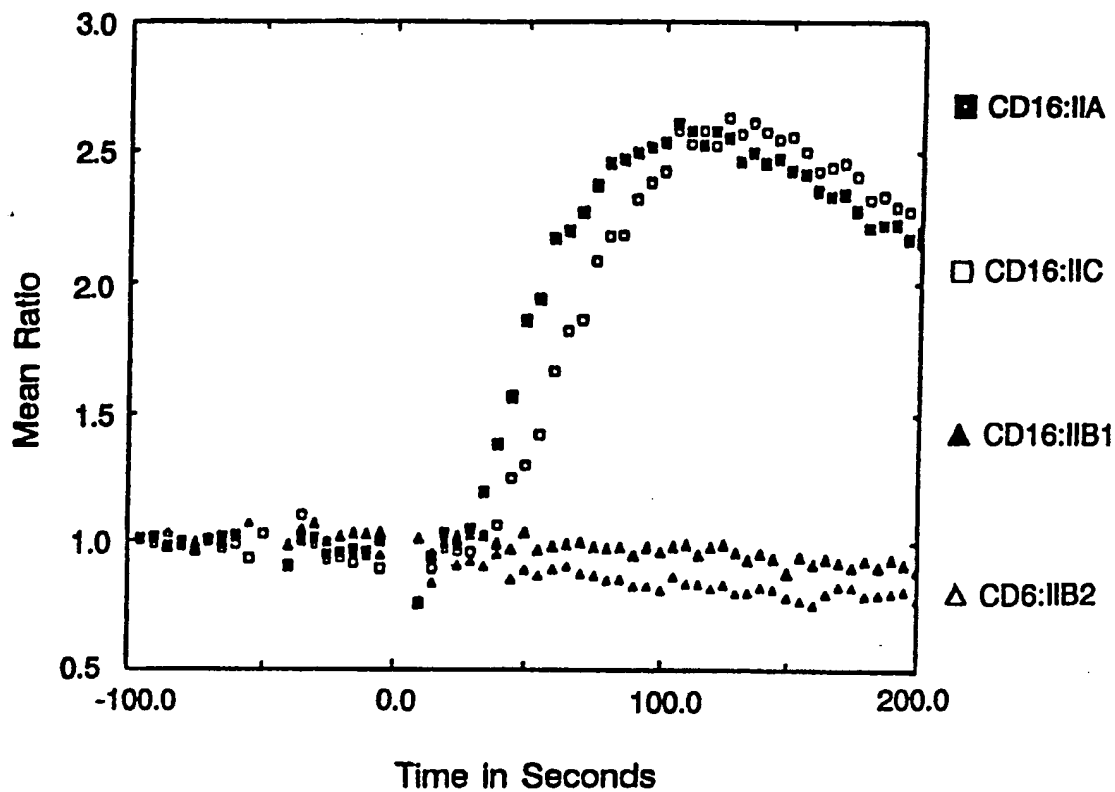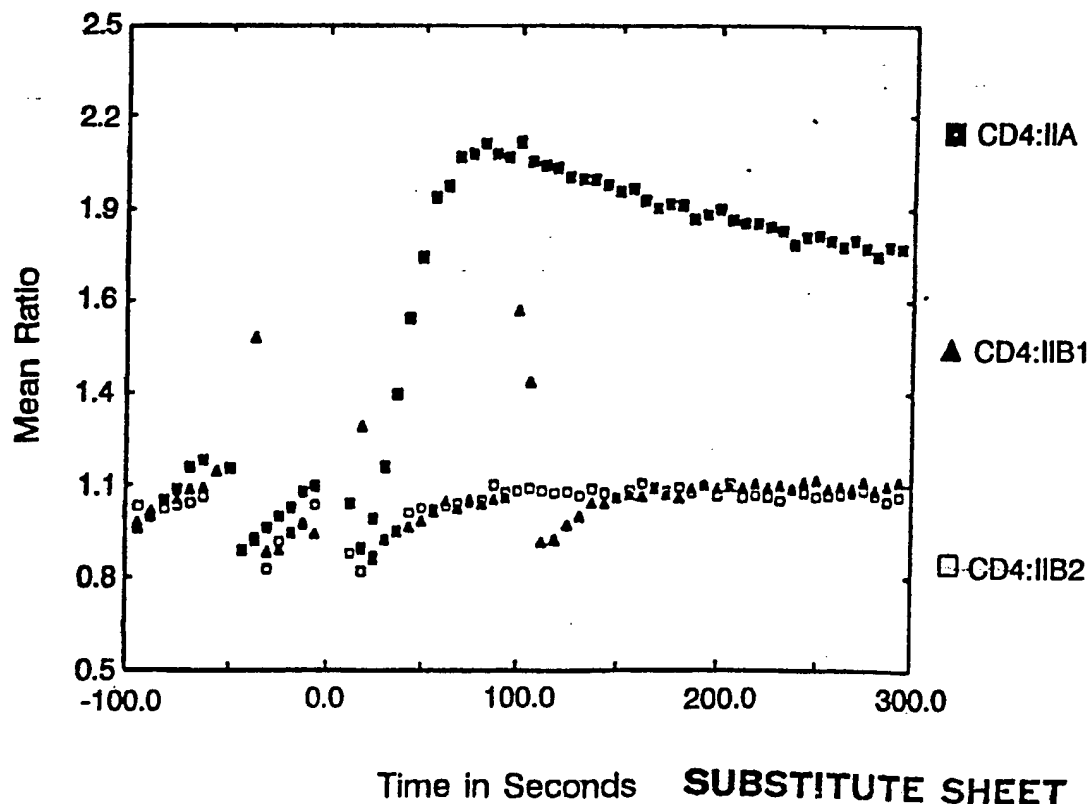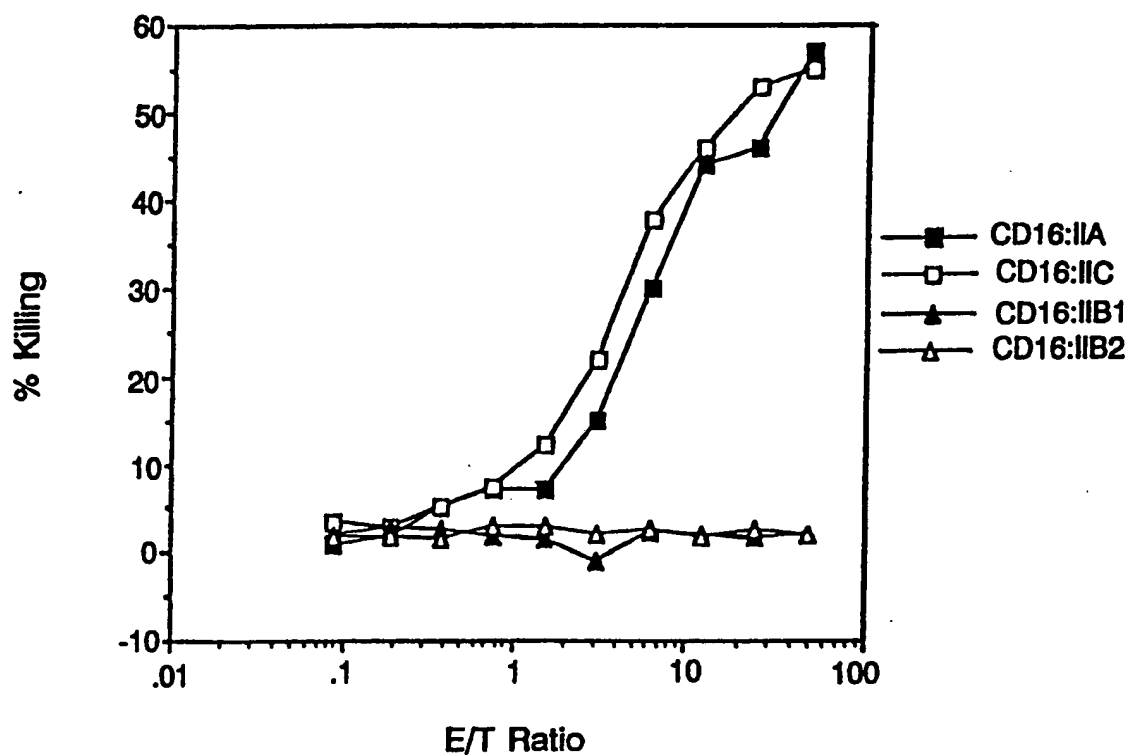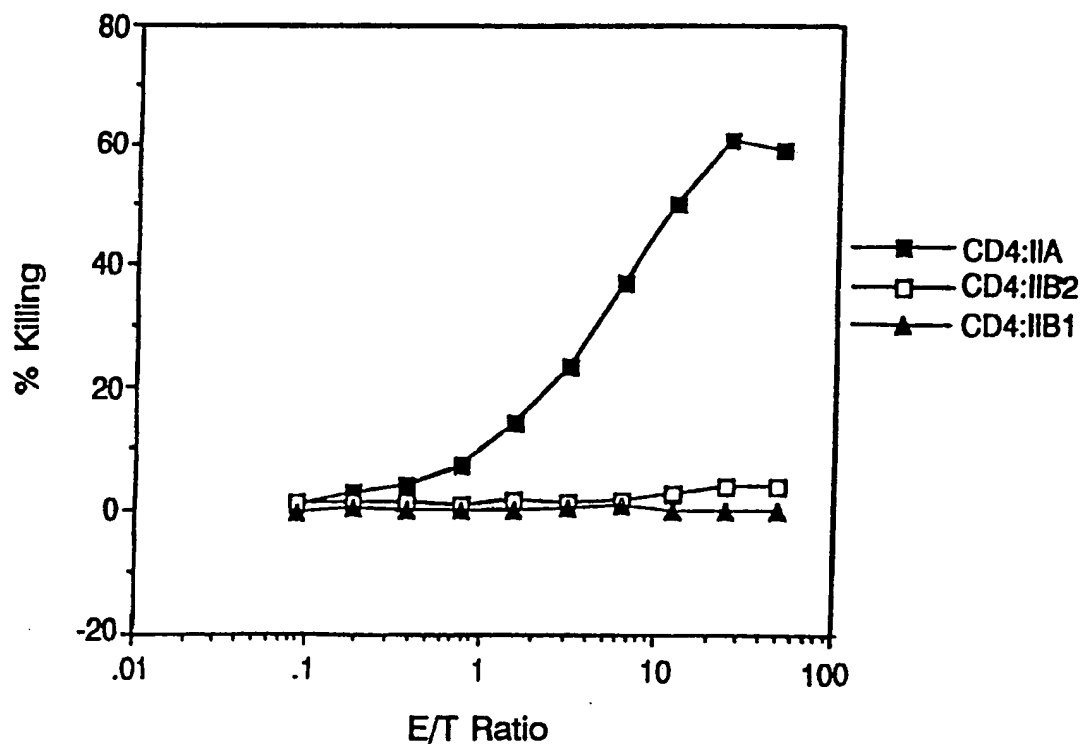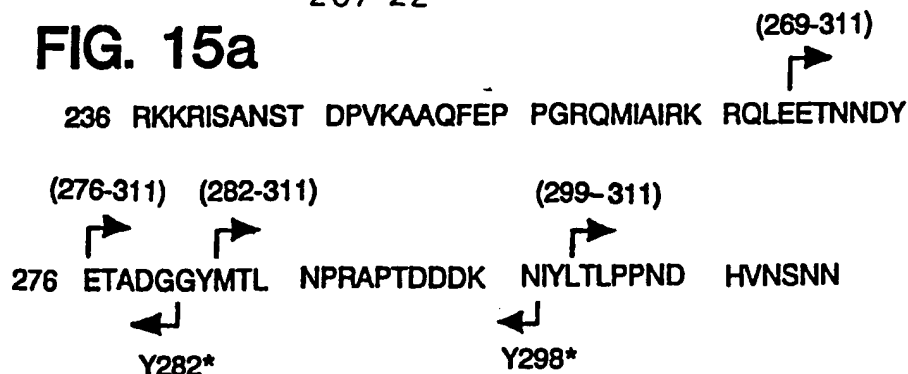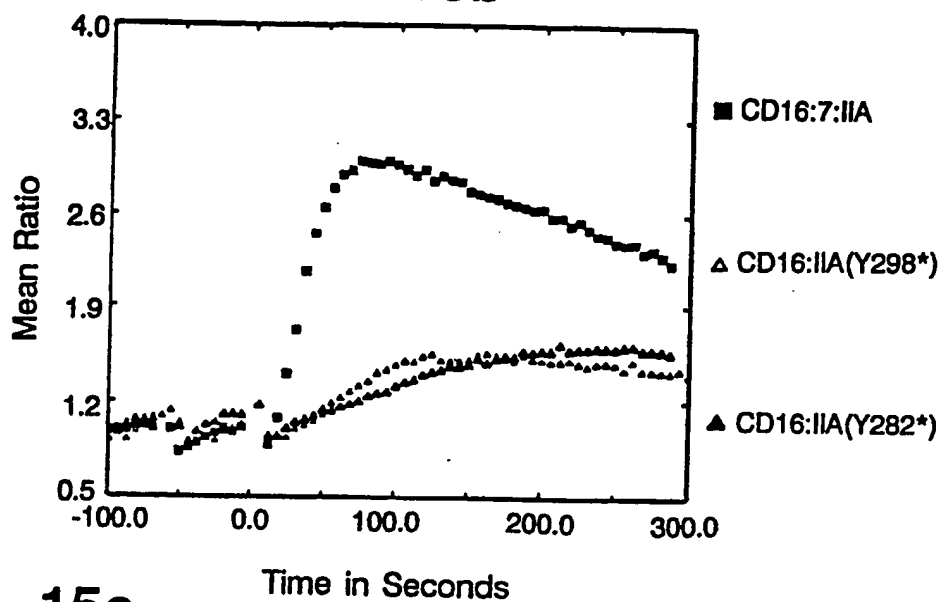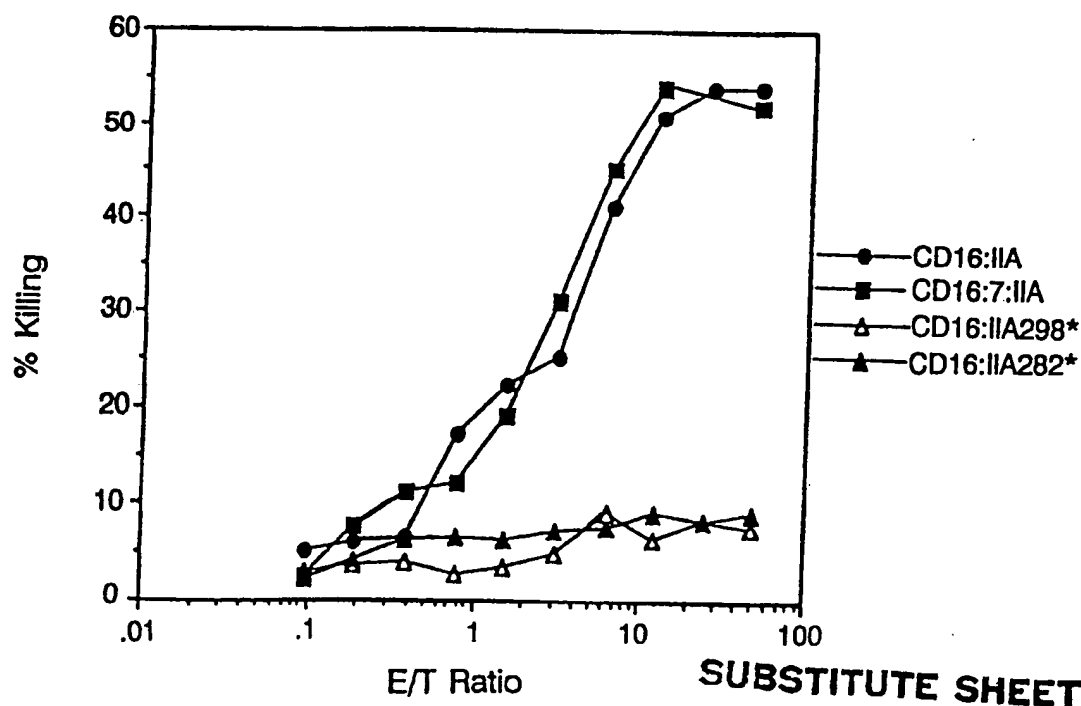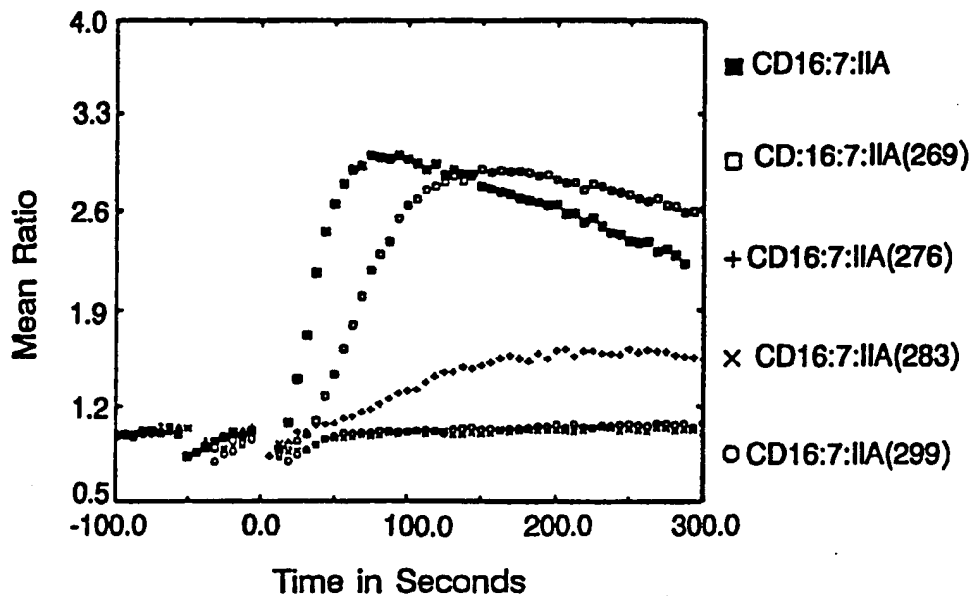
FIG. 15d

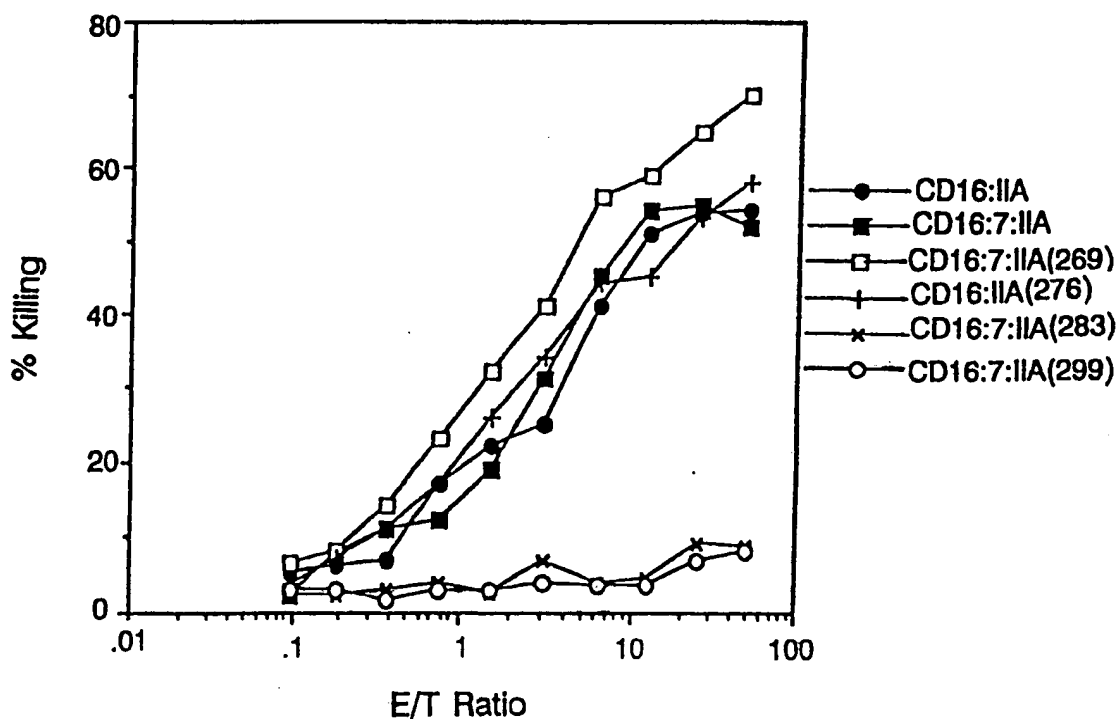# FIG. 15e

FIG.16 (Seq. ID No: 24)

```
1.   MEHSTFLSGL  VLATLLSQVS  PFKIPIEELE  DRVFVNCNTS  ITWVEGTVGT

51   LLSDITRLDL  GKRILDPRGI  YRCNGTDIYK  DKESTVQVHY  RMCQSCVELD

101  PATVAGIIVT  DVIATLLLAL  GVFCFAGHET  GRLSGAADTQ  ALLRNDQVYQ

151  PLRDRDDAQY  SHLGGNWARN  K*
```

FIG.17 (Seq ID NO: 25)

```
1    MEQGKGLAVL  ILAIILLQGT  LAQSIKGNHL  VKVYDYQEDG  SVLLTCDAEA

51   KNITWFKDGK  MIGFLTEDKK  KWNLGSNAKD  PRGMYQCKGS  QNKSKPLQVY

101  YRMCQNCIEL  NAATISGFLF  AEIVSIFVLA  VGVYFIAGQD  GVRQSRASDK

151  QTLLPNDQLY  QPLKDREDDQ  YSHLQGNQLR  RN*
```

FIG.18 (Seq ID No: 26)

```
1    MPGGLEALRA  LPLLLFLSYA  CLGPGCQALR  VEGGPPSLTV  NLGEEARLTC

51   ENNGRNPNIT  WWFSLQSNIT  WPPVPLGPGQ  GTTGQLFFPE  VNKNTGACTG

101  CQVIENNILK  RSCGTYLRVR  NPVPRPFLDM  GEGTKNRIIT  AEGIILLFCA

151  VVPGTLLLFR  KRWQNEKFGV  DMPDDYEDEN  LYEGLNLDDC  SMYEDISRGL

201  QGTYQDVGNL  HIGDAQLEKP  *
```
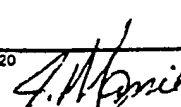
FIG.19 (Seq ID No: 27)

```
1    MATLVLSSMP  CHWLLFLLLL  FSGEPVPAMT  SSDLPLNFQG  SPCSQIWQHP

51   RFAAKKRSSM  VKFHCYTNHS  GALTWFRKRG  SQQPQELVSE  EGRIVQTQNG

101  SVYTLTIQNI  QYEDNGIYFC  KQKCDSANHN  VTDSCGTELL  VLGFSTLDQL

151  KRRNTLKDGI  ILIQTLLIIL  FIIVPIFLLL  DKDDGKAGME  EDHTYEGLNI

201  DQTATYEDIV  TLRTGEVKWS  VGEHPGQE*
```

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[3]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC (5): A61K 37/12; C07K 3/00, 13/00, 15/00, 17/00
US CL : 530/387

II. FIELDS SEARCHED

Minimum Documentation Searched[4]

Classification System	Classification Symbols
U.S.	530/387

Documentation Searched other than Minimum Documentation to the extent that such Documents are included in the Fields Searched[5]

APS, Dialog, Intelligenetics

III. DOCUMENTS CONSIDERED TO BE RELEVANT[14]

Category[*]	Citation of Document,[16] with indication, where appropriate, of the relevant passages[17]	Relevant to Claim No.[18]
X,P	Cell, Vol. 64, issued 08 March 1991, C.Romeo & B. Seed, "Cellular immunity to HIV activated by CD4 fused to T cell or Fc receptor polypeptides", pages 1037-1046, see entire document.	1-22
Y	Proc. Natl. Acad. Sci. USA, Vol. 85, issued December 1988, A.M. Weissman et al., "Molecular cloning and chromosomal localization of the human T-cell receptor zeta chain: Distinction from the molecular CD3 complex",pages 9709-9713, see entire document.	1-22
Y	J. Biol. Chem., Vol 264, No. 35, issued 15 December 1989, S.A. Carr et al., "Protein and carbohydrate structural analysis of a recombinant soluble CD4 receptor by mass spectrometry", pages 21286-21295, see entire document.	1-22
Y	Proc. Natl. Acad. Sci. USA, Vol. 83, issued May 1986, P. van den Elsen et al., "Exon/intron organization of the genes coding for the δ chains of the human and murine T-cell receptor/T3 complex", pages 2944-2948, see entire document.	1-22

* Special categories of cited documents:[15]

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search[2]	Date of Mailing of this International Search Report[2]
03 June 1992	12 JUN 1992

International Searching Authority[1]	Signature of Authorized Officer[20]
ISA/US	KAREN COCHRANE CARLSON, PH.D.

FURTHER INFORMATI N CONTINUED FROM THE SECOND SHEET		
Y	DNA and Cell Biology, Vol. 9, No. 5, issued 1990, G. Zettlmeissl et al., "Expression and characterization of human CD4: Immunoglobulin fusion proteins", pages 347-353, see entire document.	1-22
Y	EMBO J, Vol. 6, No. 10, issued 1987, A. Tunnacliffe et al., "Physical linkage of three CD3 genes on human chromosome 11", pages 2953-2957, see entire document.	1-22

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE[1]

1. ☐ Claim numbers _, because they relate to subject matter (1) not required to be searched by this Authority, namely:

2. ☐ Claim numbers _, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out (1), specifically:

3. ☐ Claim numbers _, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING[2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Search Authority did not invite payment of any additional fee.

Remark on protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (supplemental sheet(2))(Rev. 4-90) ß

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category*	Citation of Document,[16] with indication, where appropriate, of the relevant passages [17]	Relevant to Claim No.[18]
Y	EMBO J, vol. 5, No. 8, issued 1986, G.W. Krissansen et al., "Primary structure of the T3 gamma subunit of the T3/T cell antigen receptor complex deduced from cDNA sequences: evolution of the T3 gamma and delta subunits", pages 1799-1808, see entire document.	1-22
Y	EMBO J, Vol. 7, No. 11, issued 1988, N. Sakaguchi et al., "B lymphocyte lineage-restricted expression of MB-1, a gene with CD3-like structural properties", pages 3457-3464, see entire document.	1-22
Y	Proc. Natl. Acad. Sci. USA, Vol. 85, issued September 1988, G.G. Hermanson et al., "B29: A member of the immunoglobulin gene superfamily exclusively expressed on B-lineage cells", pages 6890-6894, see entire document.	1-22
Y	Proc. Natl. Acad. Sci. USA, Vol. 87, issued May 1990, Y. Jin et al., "Molecular cloning of the CD3 eta subunit identifies a CD3 zeta-related product in thymus-derived cells", pages 3319-3323, see entire document.	1-22
Y	J. Biol. Chem., Vol. 265, No. 11, issued 15 April 1990, H. Kuster et al., "Characterization and expression of the gene for the human Fc receptor gamma subunit", pages 6448-6452, see entire document.	1-22